# Evaluation of Technologies for Production of Edible Snack Foods with Demonstrated Health Benefits from Common Beans (*Phaseolus vulgaris* L.)


BY

Alex Amorim Anton


A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE


Department of Food Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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## FOREWARD

This thesis has been prepared using a paper style format. It is composed of four papers prepared for journal publication (Chapters 3, 4, 5 and 6) as well as a literature review (Chapter 2) and introduction (Chapter 1), discussion (Chapter 7), conclusions (Chapter 8) and references. Chapters 3, 4, 5 and 6 are four papers presented as originally submitted for publication except for formatting changes aimed to standardize the paper style format of this thesis.

Chapter 3, "Effect of Pre-dehulling Treatments on Some Nutritional and Physical Properties of Navy and Pinto Beans (*Phaseolus vulgaris* L.)" was published in LWT – Food Science and Technology, Issue 41, Pages 771-778, 2008 with authorship by Anton, A.A., Ross, K.A., Beta, T., Fulcher, R.G., and Arntfield, S.D.

Chapter 4, "Influence of Added Bean Flour (*Phaseolus vulgaris* L.) on Some Physical and Nutritional Properties of Wheat Flour Tortillas" was published in Food Chemistry, Issue 109, Pages 33-41, 2008 with authorship by Anton, A.A., Ross, K.A., Lukow, O.M., Fulcher, R.G., and Arntfield, S.D.

Chapter 5, "Shelf Stability and Sensory Properties of Flour Tortillas Fortified with Pinto Bean (*Phaseolus vulgaris* L.) Flour: Effects of Hydrocolloid Addition" was submitted for publication to LWT – Food Science and Technology, March 10 2008 with authorship by Anton, A.A., Lukow, O.M., Fulcher, R.G., and Arntfield, S.D.

Chapter 6, "Physical and Nutritional Impact of Fortification of Corn Starch-based Extruded Snacks with Common Bean (*Phaseolus vulgaris* L.) Flour: Effects of Bean

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# ABSTRACT

This work was undertaken to add value to local bean crops by determining the technical feasibility of utilizing common bean flour from different cultivars for the nutritional fortification of snack foods such as flour tortillas and corn starch-based extrudates. Additionally, the dehulling of beans aiming the separation of different components such as seed coats and cotyledons was also studied.

The effect of bean cultivar on some physical and nutritional properties of wheat- and corn-based snacks was more significant for substitutions higher than 25%. Fortification at 25-30% produced acceptable textural parameters and improved nutritional profile in comparison to the wheat and corn starch controls. Colour and levels of phenolics and antioxidant activity were not changed to a great extent in materials to which navy bean flour was added, however these parameters were significantly affected for the other type of beans. Levels of antinutritional compounds were significantly reduced during processing, indicating that bean snacks could be safely used for human nutrition.

# TABLE OF CONTENTS

**LIST OF FIGURES**

## LIST OF TABLES

# 1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a traditional food in the human diet, as it is low in fat and rich in proteins, vitamins, complex carbohydrates and minerals. In addition to contributing nutritional requirements, consumption of dry beans has been linked to reduced risk of heart disease (Anderson et al., 1984), obesity (Geil & Anderson, 1994) and cancer (Garcia-Gasca et al., 2002; Azevedo et al., 2003).

Economically, beans are an important crop in North America, since their production and export has increased significantly in recent years. In Canada, they are among the most produced and consumed pulses. Manitoba is the province which produces the most beans, with the main bean types being navy, pinto, and black. Lower levels of kidneys, cranberries, small red and others are also produced. Canada exports about 90% of its beans crops, suggesting that the value of such crops relies primarily on the export of clean, raw material (Agriculture and Agri-Food Canada, 2006).

Consumption of coloured beans may play an important role against oxidative stress due to the presence of polyphenols that possess *in vitro* antioxidant activity (Madhujith & Shahidi, 2005). These compounds have been promoted by health authorities, thus stimulating the consumption of foods rich in antioxidants. Increasing the supply of antioxidants in staple foods such as bread, tortillas, and snacks may provide a safety net for those who cannot or do not want to consume fruit and vegetables.

Because of their nutritional and health promoting properties, the development of value-added bean-based products for new market opportunities in the functional food and nutraceutical industry is being promoted (Singh, 1999). In this context, the use of isolated bean hulls as an ingredient for novel food products featuring high dietary fibre and high antioxidant levels appears promising, in particular for the ready-to-eat and snack food markets.

In the scientific literature several pre-dehulling treatments including soaking and/or heating have been described (Deshpande et al, 1982; Singh et al., 1992; Anderson et al., 1994), however little is known about the possible changes in the general properties of the seed coats and whole seeds of beans submitted to these processes. Additionally, attempts aimed to improve the nutritional profile of bread and corn-based snacks by adding hulls and cotyledons of legumes have been reported (Doxastakis et al., 2002; Dalgetty & Baik, 2006), nonetheless few publications have focused on improving the nutritional profile of wheat-based flour tortillas and corn-based snacks with common bean flour.

This work was undertaken to add value to local bean crops by determining the technical feasibility of utilizing common bean flour from different cultivars for the nutritional fortification of snack foods such as flour tortillas and corn starch-based extrudates. The effect of added bean flour on some physical and nutritional properties of both tortillas and extruded snacks was investigated in terms of dough rheology, texture determined instrumentally, general physical characteristics, levels of crude protein, total phenols, antioxidant activity, and antinutritional factors such as phytic acid and trypsin inhibitors. Additionally, the dehulling of beans aimed at the separation of different components such as seed coats and cotyledons was also studied, verifying the impact of various pre-dehulling treatments on the physical and nutritional properties of individual bean components.

It is expected that levels of protein, phenolics, and antioxidants will increase in finished products added of bean flour as a function of rate of substitution and bean cultivar. In comparison to wheat flour and corn starch alone, levels of antinutritional factors are also expected to increase in the raw composite flours containing bean flour, however thermal treatment is known to effectively inactivate such compounds.

# 2. LITERATURE REVIEW

## 2.1. The Common Bean

The common bean (*Phaseolus vulgaris* L.) is a member of the Leguminosae family, tribe Phaseoleae, subfamily Papilionoideae. The family Leguminosae is named after the characteristic pod or legume that protects the seed while they are forming or ripening. With approximately 13,000 species, this family is the second largest in the plant kingdom and is very important economically (Takeoka et al., 2003). Beans are the seeds or pods of plants that belong to this family. They were originally a crop of the New World, but are now grown throughout all major continental areas (Graham & Ranalli, 1997). Together with maize and cassava, beans have been a dominant staple in the low to mid-altitudes of the Americas for centuries (Broughton et al., 2003). The domestication of beans in the upland regions of Latin America dates back more than 7000 years (Gepts & Debouck, 1991).

Beans are considered the most important grain legumes for direct human consumption in the world (Broughton et al., 2003). They are usually grown in tropical countries for dry seeds and in temperate regions for dry seeds and fresh pods, which are commonly consumed fresh or processed as frozen vegetables (Fageria, 2002). Dry beans also belong to the group of pulses, which are defined by the Food and Agricultural Organization of the United Nations (FAO) as annual leguminous crops yielding from one to twelve grains or seeds of variable size, shape and colour within a pod. The term pulses, as used by the FAO, is used only for crops harvested exclusively for dry grains, excluding green beans and green peas, which are considered vegetable crops. Also excluded are crops for which the main commercial value is in their oil content (oilseeds like soybeans and peanuts), and crops which are used exclusively for sowing (clovers, alfalfa). Pulses include dry peas, chickpeas, black-eyed peas, pigeon peas, lentils, lupins, winged beans, dry broad beans, and dry beans (Tharanathan & Mahadevamma, 2003). Kidney bean, pinto bean, black bean, navy bean, small red bean, and cranberry bean are different genotypes of the common dry bean (*Phaseolus vulgaris* L.).

Total global production of dry beans exceeds 23 million metric tones, of which 7 million come from Latin America and Africa. Compared to chickpeas, which is the second most important grain legume, worldwide bean production is almost twice as high (Broughton et al., 2003). In North America, which accounts for 11.6% of world dry bean production, beans are also considered a relevant crop. Based on statistics from Agriculture and Agri-Food Canada (2006), there was a 320% increase in bean production (from 73kt to 304kt) between the 1992-1993 crop-year and the forecast for the 2005-2006 crop-year in Canada. Manitoba is the province which produces the most beans, with the main bean types being navy, pinto, and black. Lower levels of kidneys, cranberries, small red and others are also produced. Canada exports about 90% of its beans crops, suggesting that the value of such crops relies primarily on the export of clean, raw material (Agriculture and Agri-Food Canada, 2006).

In North America and many Asian countries, beans are also an important component of sustainable agriculture, since like other pulse crops they play a key role in crop rotation due to their ability to fix nitrogen. In Latin America and Eastern Africa, they are regarded as a major source of dietary protein (Graham & Ranalli, 1997). Dry bean is the principal food legume for over 500 million people in these developing regions, and for more than 20% of these people it is the leading source of dietary protein (Fageria, 2002). Nutritionally, beans are a rich and inexpensive source of proteins, carbohydrates, dietary fibres, minerals and vitamins (Rehman et al., 2001). The high nutritional quality of beans in terms of percentage protein is an important complement to starchy foods such as maize, plantains and root crops. Additionally, the high levels of minerals such as iron and zinc in the seeds of dry beans are of significant relevance in regions where there is a high prevalence of micronutrient deficiencies, including iron deficiency anemia (Broughton et al., 2003).

### 2.1.1. Basic Nutritional Value

Similar to other beans, the common bean is high in starch, protein (20-25%) and dietary fibre (18-22%), as well as an excellent source of iron, vitamins, and folic acid. (Table 2.1) (UDSA, 2007). The dietary protein from beans play an essential role in human nutrition by complementing other foods that are carbohydrate-based (Broughton et al., 2003). Much of the dry beans proteins are made up of the storage protein phaseolin (Ma & Bliss, 1978), which is an important determinant of both quantity and quality of proteins in bean seeds (Gepts & Bliss, 1984; Broughton et al., 2003). In the legume family, proteins are deficient in sulphur-containing amino acids such as methionine. Cereals, however, generally contain sufficient sulphuryl amino acids but are low in other essencial amino acids such as lysine.

**Table 2.1.** Nutritional Value of Raw Kidney Beans*

| Nutrient | Units | Value per 100 grams |
|---|---|---|
| Water | g | 11.75 |
| Energy | Kcal | 337 |
| Protein | g | 22.53 |
| Total Lipid (Fat) | g | 1.06 |
| Ash | g | 3.37 |
| Carbohydrate, by difference | g | 61.29 |
| Fibre (total dietary) | g | 15.20 |
| Sugars, total | g | 2.10 |
| Phantothenic acid (B5) | mg | 0.78 |
| Folate (B9) | mcg | 394 |
| Calcium | mg | 83 |
| Iron | mg | 6.69 |
| Magnesium | mg | 138 |
| Zinc | mg | 2.79 |
| Lysine | g | 1.55 |
| Methionine | g | 0.34 |

*Adapted from the USDA National Nutrient Database for Standard Reference, Release 20 (2007) (USDA, 2007).

Combining the consumption of cereals and legumes is a valuable approach of assuring a diet included of all essential amino acids (Bressani, 1983). In bakery goods, addition of legume flour to cereal-based formulations has proven to positively impact their essential amino acid balance (Koehler et al., 1987; Shehata et al., 1988; Tharanathan et al., 2003).

Compared to cereals, legumes are far more superior as a source of micronutrients (Welch et al., 2000). This makes sense on the basis that legumes, apart from having a higher initial content of minerals, are not polished before eating, which is the case of many cereals. Since minerals are mostly concentrated in the seed coats (or bran) of cereals, discarding of these parts during processing causes the abrupt decrease in the mineral content of the refined material. In beans, which are commonly consumed whole, such a negative effect is not observed. Beans are an important source of iron, phosphorus, magnesium, manganese, and to a lesser extent, zinc, copper, and calcium (Broughton et al., 2003). Although it appears that beans would qualify as an extraordinary food, their widespread use as a primary staple food has been limited by the presence of antinutritional factors, which might produce adverse effects for human and animal nutrition.

### 2.1.2. Antinutritional Factors

Antinutritional factors present in dry beans include enzyme inhibitors (e.g. trypsin inhibitors), lectins, phytates (or phytic acid), cyanoglycosides and phenolics (Martin-Cabrejas et al., 2004). While trypsin inhibitors have been reported to be thermolabile and therefore easily inactivated during thermal processing, phytic acid is known to be quite stable in thermal treatments, undergoing only partial hydrolysis (Estévez et al., 1991; Abd El-Hady & Habiba, 2003; Rehman & Shah, 2005).

Phytic acid and their salt (hexaphosphate myoinositol) are the main reserve of phosphate in plants (Urbano et al., 2000). In animal and human nutrition, phytic acid chelates various divalent metal ions and is involved in their reduced absorption leading to deficiency symptoms (Broughton et al., 2003). Conversely, it has been recently reported that phytates might have possible therapeutic properties, in particular in the prevention of cancers of the breast and colon. This was attributed to their antioxidant properties. It has been also related to reduced cholesterol and other lipids due to its presence in diets high in fibre (Thompson & Zhang, 1991).

In legumes, phytates are associated with protein bodies (Reddy et al., 1982), thus phytic acid levels should increase with increasing protein content in such foods. This compound has been reported to be resistant to thermal treatments however reductions in phytic acid levels in beans have been explained on the basis that during cooking inositol hexaphosphate could have been hydrolyzed to lower molecular weight forms (Alonso et al., 2000).

Of the protease inhibitors present in legumes, the most important are the trypsin inhibitors, whose action has been thoroughly studied (Deshpande et al., 1983; Estévez et al., 1991; Adb El-Hady et al., 2003). Trypsin inhibitors reduce the bio-availability of trypsin, an enzyme essential to the nutrition of many animals, including humans. Reducing trypsin bio-availability, an enzyme critical for protein digestion, may lead to a state of malnutrition due to decreased protein digestibility (Tharanathan & Mahadevamma, 2003). These inhibitors are thermolabile and their inhibitory activity can be reduced considerably by an appropriate thermal treatment (Alonso et al., 2000; Shimelis et al., 2007). Extrusion cooking of beans has been shown to eliminate almost all the trypsin inhibitor activity. Balandrán-Quintana et al. (1998) and Alonso et al. (2000) observed that extrusion cooking was one of the best processing methods for improving the protein quality of legumes.

Additionally, some publications on *P. vulgaris* have focused on antinutritional aspects of seed coat polyphenols (Elias et al., 1979; Barampama & Simard, 1993). However, polyphenols have contradicting positive effects on human health and it has been reported that they have anti-carcinogenic and antioxidant properties (Gamez et al., 1998).

### 2.1.3. Phenolics and Antioxidants

Recently, antioxidant activity was reported in extracts, condensed tannins and pure flavonoids from coloured genotypes of common bean seed coats (Beninger & Hosfield, 2003; Madhujith & Shahidi, 2005). Antioxidants in beans are related to the presence of phenolic compounds that influence their seed coat colour (Beninger et al., 2003; Madhujith et al., 2003). In this regard, coloured dry beans such as red, pinto and black, are expected to possess stronger antioxidant activity than navy beans. Coloured dry beans have higher concentration of phenolic compounds, such as flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins) in the seed coat (Feenstra, 1960) than navy beans.

According to Madhujith et al. (2005) and Espinosa-Alonso et al. (2006), while coloured dry beans may be an important source of dietary antioxidants, the method of determination of antioxidant activity plays an important role in the quantification of antioxidant capacity of these foods. Xu and Chang (2008) reported that the oxygen radical absorbance capacity (ORAC) is the only method so far that combines both inhibition time and degree of inhibition into a single quantity (Cao & Prior, 1999). The antioxidant reaction mechanism of ORAC is quite different than that of 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$). ORAC reactions involve a hydrogen atom transfer mechanism, while $DPPH^{\bullet}$ mechanism involves a single electron transfer (Prior et al., 2005). In the ORAC, antioxidant activity provokes the inhibition of the free radical damage to the fluorescent compounds. Different antioxidant activity values have been reported for the same food evaluated through different methods such as $DPPH^{\bullet}$, ORAC, and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) (Sánchez et al., 2007; Wang & Ballington, 2007). Arnao (2000) speculated that lower DPPH values may be attributed to the interference of other pigments that also absorb at the wavelength used in the $DPPH^{\bullet}$ method (515 nm), such as carotenoids and anthocyanins (Dlamini et al., 2007).

Additionally, levels of polyphenols and antioxidant activities of common beans appear to be significantly affected by thermal treatments (Alonso et al., 2000; Korus et al., 2007a; Korus et al., 2007b). Boiling, roasting, microwaving, and extrusion cooking have been reported to either increase or decrease the antioxidant capacity of beans (Alonso et al., 2000; Stewart et al., 2000; Abd El-Hady & Habiba, 2003; Turkmen et al., 2005). To date it remains unclear what mechanisms are responsible for such changes.

Nonetheless, these compounds have been promoted by health authorities, thus stimulating the consumption of foods rich in antioxidants (USDA, 2005). Increasing the supply of antioxidants in staple foods such as bread and tortillas may provide a safety net for those who cannot or do not want to consume fruit and vegetables. As a result of this trend, attempts to fortify commonly consumed white bread with antioxidants have been reported (Park et al., 1997a; Park et al., 1997b). Antioxidants are credited with scavenging free radicals and reactive oxygen species, playing an important role against oxidative DNA damage and cellular transformation that lead to degenerative diseases (Madhujith & Shahidi, 2005).

Aside of being low in fat and rich in proteins, vitamins, complex carbohydrates and minerals, consumption of dry beans has been linked to reduced risk of heart disease (Anderson et al., 1984; Winham & Hutchins, 2007), obesity (Geil & Anderson, 1994) and cancer (Garcia-Gasca et al., 2002; Azevedo et al., 2003).

### 2.1.4. Post-Harvest Processing for Food Uses

Storage and processing of foods are technologies that serve as prerequisites for insuring availability of the food supply as well as improving the quality of certain types of food. This includes increasing stability, flavour improvement, reduction of toxicity, and development of functionality (Singh, 1999).

After harvest, common beans often contain a high amount of moisture that has to be removed before storage. In developing countries, this is usually done by exposing the seeds to solar radiation. It is noteworthy that excessive time of exposure may increase the possibility of the seeds developing the hard-to-cook phenomenon. Hard-to-cook seeds can also develop during poor storage conditions, which mainly concerns storage temperature and time, and relative humidity. Such seeds have proven to have lower nutritional value and low consumer acceptability (Singh, 1999).

Common processing methods for common beans include milling, germination, fermentation, soaking, and cooking. All these procedures will cause important changes in the physical and nutritional properties of beans. Cooking is certainly the most common practice used worldwide for the utilization of beans as a food. It includes roasting, pressure cooking, boiling, and extrusion, among other methods (Elías et al., 1979; Singh, 1999).

### 2.2. Tortillas

Flour tortillas are a unique baked product that has been produced in Mexico for centuries. Tortillas are now more popular in United States than bagels, croissants, English muffin, pitas, or any other type of ethnic bread. In 2005, the baking industry in United States showed a consistent increase in the flour tortilla market. While the consumption of fresh bread was up 0.3%, the increase in tortilla sales was up 3.5% in comparison to the previous year (Kuk, 2006). In the "State of the Tortilla Industry Survey: 2000", it was reported that U.S. sales at wholesale prices for tortillas totaled more than US$ 4 billion in 2000, representing a growth rate of 57% over the previous four years (Tortilla Industry Association, 2007).

The major ingredient for production of flour tortillas is wheat flour, while corn tortillas are produced from lime-cooked, stone-ground corn. Although flour tortillas are produced with many of the same ingredients as bread, processing technologies and product characteristics are quite different (Dally & Navarro, 1999). A flour tortilla can be defined as a flat, circular, light-coloured bread. Usually they have an average thickness of 1/16 in (16 mm) and diameters ranging from 6 to 13 in (15 to 33 cm). They are generally eaten with beans, meats, cheese, avocados, spreads, and other ingredients (Waniska, 1999).

As tortilla is the fastest growing segment of the North American baking industry (Cornell, 1998; Kuk, 2006; Tortilla Industry Association, 2007), reconstructing the tortilla with new nutritional attributes and in new formats has been part of this growth as have changes in the tortilla industry itself. On the other hand, the increasingly popular market for functional foods and the search for healthier alternatives to conventional foods, with the consumer desire for convenience and practicability, has also increased (Berne, 2005).

Nutritionally, flour tortillas are rich in carbohydrates that generate a high glycemic response after ingestion, similar to white bread (Saldana & Brown, 1984). Hence, formulating more nutritious tortillas, with more protein, dietary fibre and antioxidants, would be well received by consumers. Additionally, food scientists should also focus on increasing the shelf life of tortillas. While consumers typically reject bread after one week on the grocery shelf, they expect tortillas to be edible over weeks and even months at a time (Friend et al., 1995). Various tortilla formulations include hydrocolloids to extend shelf life and retain freshness. Hydrocolloids are water-soluble polysaccharides with varied chemical structures providing a range of functional properties that make them suitable for shelf life extension.

### 2.2.1. Processing of Flour Tortillas

Tortillas are produced by three basic methods: hot pressing, die cutting, and hand stretching (Figure 2.1). Since the 1970's, when Mexican fast food chains started to operate on a large scale in Canada and United States, tortilla production has increased about tenfold (Lind & Barham, 2004). More than 90% of the increase is represented by tortillas made by the hot-press method (Waniska, 1999).

In the hot-press method, rested and relaxed dough pieces are transferred onto a heated conveyor plate where a hydraulic press device is typically used to form discs from dough balls. During pressing a thin skin is formed, which helps to seal the tortilla and limits the release of steam and carbon dioxide generated during baking. This contributes the typical puffed characteristic of the tortilla. In general, hot-pressing is not very efficient, but results in tortillas with the desired soft texture and better retention of flexibility during storage. These tortillas are well suited for fajitas, soft tacos, burritos and wraps (Dally & Navarro, 1999; Waniska, 1999).

The die-cut method is more efficient, yielding a lower cost product. However, the quality of the resulting tortillas is inferior to the hot-press counter-parts. They are less soft, more pasty, and lose flexibility more quickly. Most die-cut tortillas are used to prepare processed foods, such as burritos, chimichangas, or enchiladas. Few are merchandised as tortillas on the retail market (Waniska, 1999). They are produced by dough extrusion and gradual multi-step sheeting to the desired thickness. Dough sheets are then passed under a die-cut cylinder and to produce uniformly shaped products (Dally & Navarro, 1999).

The hand-stretch method usually produces tortillas that are larger, thinner, and stronger than pressed or die-cut approaches. The relaxed dough pieces are passed through a pair of sheeting rolls to form an elongated shape and passed again through another pair of rolls at a 90° angle to shape them into round, flat discs. They are then hand-stretched to the final shape and diameter (Dally & Navarro, 1999). Although the characteristics of the final product are acceptable, the increased labor and the slower rate of production discourage most manufacturers from utilizing this method (Serna-Saldivar et al., 1988).

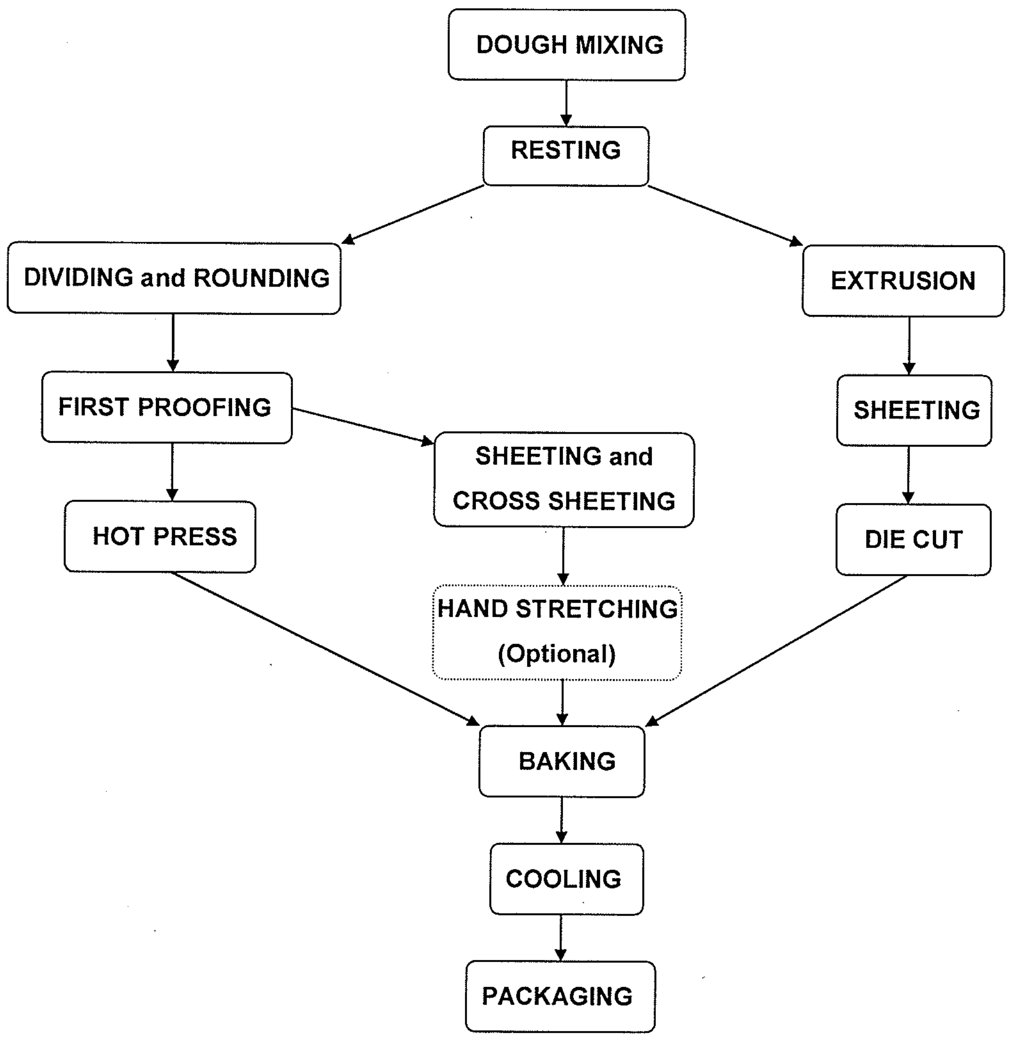


**Figure 2.1**. General Schemes for the Production of Flour Tortillas by Hot-press, Hand-stretch, and Die-cut Methods (Adapted from Serna-Saldivar et al., 1988)

After the raw tortilla discs are produced by any of the three methods, they are baked in a three-level tortilla oven. Oven temperatures and baking times are adjusted according to the desired characteristics of the end-product. Fresh baked tortillas are cooled on multilevel conveyors before packaging (Dally & Navarro, 1999).

### 2.2.2. Improving the Nutritional Profile of Flour Tortillas

Flour tortillas contain four major ingredients: flour, water, shortening/oil, and salt. In Mexico, flour tortillas contain only these ingredients, resulting in products with short shelf-life (2-4 days). However, in North America, where a far longer shelf-life is required (10-20 days), formulations contain preservatives, chemical leavening agents, emulsifiers, hydrocolloids and other ingredients to improve softness and shelf life as well as tortilla flavour and functionality (Cornell, 1998).

Generally, enriched, bleached, hard-wheat flour is utilized, but all purpose and soft-wheat flours are also applied in some operations. Oilseed flours have been traditionally used to fortify staple foods such as corn tortillas and table bread (Dhingra & Jood, 2001; Cardenas et al., 2005). Among the different types of oilseeds, soybean has been preferred due to its favorable amino acid composition which complements the amino acid profile of cereals (Dhingra & Jood, 2001).

Besides traditional ingredients, efforts to increase the nutritional value of corn and flour tortillas by incorporating alternative flours date back at least 25 years. The protein quality of corn tortillas, wheat flour tortillas, and corn-soy tortillas (90% corn: 10% soy; 80% corn: 20% soy) has been evaluated (Valencia et al., 1979). Based on parameters such as protein efficiency ratio, net protein ratio, nitrogen utilization and relative nitrogen utilization, the corn-soy mixtures (e.g. 80:20 and 90:10) had the highest protein quality. The protein quality of corn and flour tortillas was similar.

Since the dietary fibre content of flour tortillas is similar to white bread (Saldana & Brown, 1984), attempts to increase their fibre content by adding mixtures of whole and refined red or white wheat flours have been performed (Friend et al., 1992). Whole wheat flour significantly influenced the dough water absorption (increased), colour (darker) and storage stability (decreased). Furthermore, fibre content of tortillas was increased by adding either 10% oat bran or 10% rice bran; but both significantly affected dough machinability and the mixing process. The authors concluded that acceptable tortillas can be prepared from both whole red and white wheat flours, however tortillas prepared using 50% whole wheat flour were more acceptable than were tortillas prepared with 100% whole wheat flour. Additionally, it was observed that tortillas containing whole white wheat flour had higher acceptability than those with whole red wheat flour, which may be explained on the basis that the latter causes darker colour.

Conversely, Seetharaman et al. (1994) investigated the application of fibres isolated from corn, oat, pea, soy, and sugar beet in the processing of flour tortillas. Based on a consumer acceptability test, the investigators reported that tortillas containing 8% soy or oat fibres were as acceptable as the control, concluding that tortillas with a good shelf stability and high consumer acceptability could be made by adding up to 12% of some fibres.

As shown, alternative ingredients that can improve the nutritional profile of wheat flour tortillas have been successfully applied. However, as different raw materials are added to a traditional formulation the texture is significantly affected, as are the shelf-life and other sensory properties. Soluble and insoluble fibres have been employed in wheat flour tortillas showing different plausible mechanisms which lead to poorer final product quality (Seetharaman et al., 1997). Addition of soluble fibre at 8% resulted in poor gluten development and extensive starch gelatinization during baking, producing tortillas with denser crumbs. Insoluble fibres, however, were shown to physically disrupt the gluten network, causing the collapse of air bubbles and tortillas with decreased shelf-stability (Seetharaman et al., 1997).

### 2.2.3. Improving the Textural Profile of Flour Tortillas

Considered a convenient bakery good, tortillas are now expected to last for several days. Moreover, they are frequently frozen and are then expected to have the same quality characteristics as the fresh product after thawing (Cornell, 1998). In this context, the use of additional ingredients that can improve the textural characteristics of the traditional and novel flour tortillas appears very promising.

Changes to formulations including the addition of anti-microbial agents, acidulants, leavening agents, yeast, nonfat dry milk, emulsifiers and hydrocolloids have been reported (Friend et al., 1992; Friend et al., 1993; Friend et al., 1995). These additives were aimed to improve production efficiency, product uniformity, shelf-stability and texture.

The texture of bakery goods is often improved with the addition of hydrocolloids. They comprise a number of water-soluble polysaccharides with different chemical structures providing a range of functional properties that make them widely used in the food industry. Hydrocolloids are able to modify starch gelatinization (Rojas et al. 1999), and to extend the overall quality of the product during time. In addition, some studies have reported the use of hydrocolloids as fat replacers (Lucca & Trepper, 1994). Contributing to bakery goods, they act by improving shelf life stability and texture by retaining more moisture and retarding staling. There is an increasing demand for hydrocolloids in baked goods, where they have been utilized for diverse purposes.

The most important instrumentally determined physical parameters in the shelf stability and quality of tortillas are cohesiveness, rollability and moisture loss (Friend et al., 1992; Friend et al., 1993; Friend et al., 1995). The beneficial effects of hydrocolloids in tortilla processing have been discussed by Gurkin (2002). The major functions of hydrocolloids and their interactions were reviewed, and the author concluded that water-binding is the main feature of gums in tortillas. The ability of the large molecules to hold moisture was reported to help prevent staling. Moreover, the interactions between different structures were emphasized. In this regard, the author cited the beneficial combination of guar gum with CMC as water-binding agents, confirming their potential as textural improvers.

Friend and colleagues (1993) studied the addition of natural gums (Arabic, guar, and xantham), modified cellulose (CMC, HPMC and methylcellulose) and commercial blends (mixtures of natural and modified cellulose gums) of hydrocolloids on processing and qualities of flour tortillas. It was found that tortillas with added hydrocolloids were consistently round, puffed, slightly browned, and of good quality. Texture was verified by determining rollability over time, and tortillas containing CMC and cellulose-based commercial blends were reported to retain their rollability for longer. During freezing and thawing, rollability of all tortillas decreased, however those containing CMC were significantly more rollable than the control after five freeze-thaw cycles.

### 2.3. Extrusion

Extrusion cooking is an important processing technique in the food industry as it is considered an efficient manufacturing process (White, 1994). Food extruders provide thermo-mechanical and mechanical energy (shear) necessary to cause physic-chemical changes of raw materials with an intense mixing for dispersion and homogenization of ingredients including conveying, mixing, shearing, heating or cooling, shaping, venting volatiles and moisture, flavour generation, encapsulation and sterilization (Linko et al., 1981; Wiedman & Strobel, 1987). Advantages of this process is that one extruder can operate at relatively low temperatures and produce pasta and baking goods, or at very high temperatures, and then manufacture products with low bulk density, such as snacks and ready to eat cereals (Harper, 1981).

Extruded foods are composed mainly of cereals, starches, and/or vegetable proteins. The major role of these ingredients is to give structure, texture, mouth feel, bulk, and many other characteristics desired for specific finished products (Launey & Lisch, 1983; Tahnoven et al., 1998). Consumer acceptance of extruded foods is mainly due to the convenience, value, attractive appearance and texture found to be particular for these foods, especially when it concerns snack products (Harper, 1981).

### 2.3.1. Snacks from Cooking Extrusion

Snack foods comprise a very large variety of items including potato chips, crackers, nuts and extruded snacks, among others (Harper, 1981). Snack food extrusion includes subjecting selected grains to a variety of complex physical processes to yield snacks with varied shapes and textures.

In extruding snack foods, grain and other ingredients are mixed and cooked under pressure, shear and high temperature in a tube, which is also called a barrel. The resulting mass is forced through a die, after which it is cut into individual pieces and assumes the various shapes that consumers have come to expect in the snack food aisles of markets (Harper, 1981). Novel ingredients, cutting-edge extrusion technology and innovative processing methods are combined to yield new snack products with ever-widening appeal to health-conscious consumers that are seeking different textures and mouth feeling with convenience (Pamies, 2000).

In order to make products that will be acceptable in a very competitive market, extrusion of snack foods demands the control of many parameters that will directly or indirectly influence the consumer acceptability. Apart from the intrinsic properties of the raw materials (e.g. starch profile, levels of protein and fibre), finished product characteristics are partly the result of specific critical parameters induced in such raw materials. The critical parameters that will influence and characterize the moisture, expansion, solubility, absorption, colour, flavour and texture of the final product are:

- Moisture: the actual moisture in the raw mixture, can be injected during pre-mixing or added during conditioning;

- Thermal energy input: heat from thermal fluids, steam or electricity that is transferred to the heads of the extruders, or direct injection of steam or any other type of heated liquid;
- Mechanical energy input: heat dissipated into the extrudate caused by the shearing and pumping action in the extruder barrel;
- Retention time: total time the product is in any specific region of the extrusion process (Huber, 2001).

In addition, several extrusion processing conditions contribute to the quality of finished products. The control of feed rate, screw speed, barrel temperature and barrel pressure, together with the above mentioned critical parameters, will determine the crispness, hardness and various other characteristics that will influence the success of the product (Harper, 1981).

The success or failure of a new extruded snack food product is directly related to sensory attributes, where texture plays a major role. In such foods, where expansion is desired and puffed products are expected, texture is of major importance, with crispness being one of the most important attributes (Pamies et al., 2000).

While corn starch provides all the features for production of highly acceptable extruded snack foods, its nutritional value is far from satisfying the needs of health-conscious consumers (Rampersad et al., 2003). Several attempts to improve the nutritional profile of extruded starch have been reported (Liu et al., 2000; Onwulata et al., 2001; Rampersad et al., 2003). Among other materials, incorporation of legume flours caused a positive impact on levels of proteins and dietary fibre of corn starch-based extruded snacks (Berrios, 2006). On the other hand, addition of high-fibre, high-protein alternate ingredients to starch significantly affected the texture, expansion and overall acceptability of extruded snacks (Liu et al., 2000; Veronica et al., 2006). For the production of nutritious acceptable snacks, rates of starch fortification seem to vary according to the nature of each material. Legumes, for example, have been reported to cause good expansion and are, therefore, regarded as highly feasible for the development of high-nutritional, low-calorie snacks (Berrios, 2006).

## 3. Effect of Pre-dehulling Treatments on Some Nutritional and Physical Properties of Navy and Pinto Beans (*Phaseolus vulgaris* L.)

### 3.1. Abstract

The effect of pre-dehulling treatments using low and high temperatures on some nutritional and physical properties of navy and pinto beans was investigated. Beans were exposed to water (14%,28% and soaking 1:5, w/v for 6h, 16h and 16h respectively) to facilitate seed coat detachment prior to freeze-drying (FD) for 48 h or heat-drying (HT) for 20 or 60 min. Exposure to the highest moisture levels produced the largest seed coat yields (17.38-20.91%) and was independent of the drying conditions. The total phenolic content was positively correlated to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant activity and increased as the exposure time to HT increased, but it was unaffected by the FD. The nutritional properties of the bean varieties differed significantly, phytic acid was unaffected, and the highest antioxidant activity was observed on the seed coats of heat-dried pinto beans (69.24 – 84.46 % of DPPH discolourration). Significant physical changes were observed for the heat-dried seeds, with the highest yellowness and the lowest peak viscosity detected in the soaked and heat-dried (60 min) beans.

### 3.2. Introduction

Common bean (*Phaseolus vulgaris* L.) is a traditional food in the human diet, as it is low in fat and rich in proteins, vitamins, complex carbohydrates and minerals. In addition to contributing nutritional requirements, consumption of dry beans has been linked to reduced risk of heart disease (Anderson et al., 1984), obesity (Geil & Anderson, 1994) and cancer (Garcia-Gasca et al., 2002; Azevedo et al., 2003).

Economically, beans are an important crop in North America, since their production and export has increased significantly in recent years. In Canada, navy and pinto beans are among the most produced and consumed pulses (Agriculture and Agri-Food Canada, 2005). However, widespread use of beans as a primary staple food has been limited by the presence of antinutritional factors, which might produce adverse effects for human and animal nutrition. Some of these compounds include enzyme inhibitors, lectins, phytates, cyanoglycosides and phenolics (Martin-Cabrejas et al., 2004). Some publications on *P. vulgaris* have focused on antinutritional aspects of seed coat polyphenols (Elias et al., 1979; Barampama & Simard, 1993). However, polyphenols have contradicting positive effects on human health and it has been reported that they have anti-carcinogenic and antioxidant properties (Gamez et al., 1998). It is believed that antioxidants scavenge free radicals and reactive oxygen species, which can be of great importance in inhibiting oxidative mechanisms that lead to degenerative diseases (Madhujith & Shahidi, 2005). Recently, antioxidant activity was reported in extracts, condensed tannins and pure flavonoids from coloured genotypes of common bean seed coats (Beninger & Hosfield, 2003; Madhujith & Shahidi, 2005).

Because of their nutritional and health promoting properties, the development of value-added bean-based products for new market opportunities in the functional food and nutraceutical industry is being promoted (Singh, 1999). In this context, the use of isolated bean hulls as an ingredient for novel food products featuring high dietary fibre and high antioxidant levels appears promising, in particular for the ready-to-eat and snack food markets.

In the scientific literature several pre-dehulling treatments including soaking and/or heating have been reported (Deshpande et al, 1982; Singh et al., 1992; Anderson et al., 1994), however little is known about the possible changes in the general properties of the seed coats and whole seeds of beans submitted to these processes. Although the dehulling of beans without previous treatments has been reported (Ehiwe & Reichert, 1987; Cardador-Martínez et al., 2002), it seems that this process is still a technological issue for the nutraceutical and food industries, because in some varieties the seed coat is well-attached to the cotyledons.

The purpose of the current study was to examine the effect of various pre-dehulling treatments, using low and high temperatures, on some nutritional and physical properties of navy and pinto beans. Nutritionally, the content of total phenolics and its correlation with antioxidant activity, as well as the phytic acid levels were investigated. Colour and the pasting properties were determined in order to verify the possible physical changes.

## 3.3. Materials and Methods

### *3.3.1. General*

Navy (AC Mast) and pinto (Maverick) beans were obtained from the Agriculture and Agrifood Canada Research Station in Morden, MB, Canada. The cultivars were exposed to the same environmental conditions in order to avoid external variation.

To provide a control, raw beans were dehulled manually using a 38.1 mm needle without any previous treatment.

Six pre-dehulling treatments were applied to navy and pinto beans (*Phaseolus vulgaris* L.) as shown in Figure 3.1. Each treatment was performed using 300 g of dried beans.

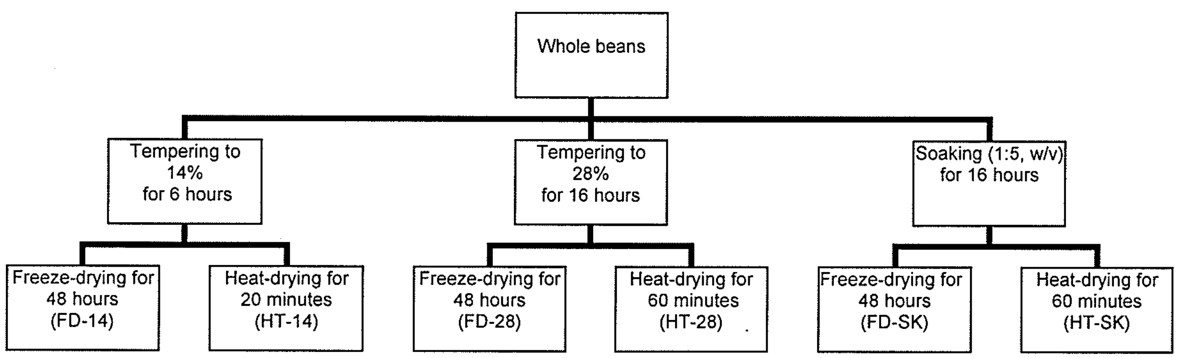


**Figure 3.1.** Flow Diagram for the Pre-dehulling Treatments of Whole Navy and Pinto Beans

For the tempering treatments, samples were mixed thoroughly with the predetermined amount of deionized water required to reach the desired tempering level. Uniformity of water absorption in the seeds was assured by tumbling the samples every 10 min for the first hour, and then once every hour for at least 4 hours.

The freeze-drying (FD) treatments were operated at −50 °C, 5 pa, for 48 h in a freeze dryer (Genesis 25 & 35 Freeze Dryer, SP Industries, USA) with samples previously frozen for 16 hrs at -40 °C.

The heat-drying (HT) treatments were performed in a hot air convection heater (S.K. Engineering & Allied Works, Model SK/LAB/PH, India), at 100 °C under manual stirrings at 10 minutes intervals.

After the pre-dehulling treatments 50 g of whole seeds were kept apart for future analysis and the remainder was submitted to the same dehulling process. This consisted of passing the beans through a shredder (S.K. Engineering & Allied Works, Model SK/SD, India) where the seed coats were removed. An aspirator (S.K. Engineering & Allied Works, Model SK/ASP, India) was then used to isolate the seed coat from the cotyledons through the adjustment of air flow. All samples were passed twice through these machines (shredder & aspirator) to improve the separations.

The fraction weights were determined and the whole seeds and the seed coats were then ground in a coffee grinder (Smart Grind, Black & Decker, USA) so that the meal passed through a 500 μm sieve (35 mesh US Standard Sieve Series). The ground samples were stored at 5 °C for no more than 3 weeks in opaque, closed containers. Chemical analyses were performed on ground samples only after they were warmed to room temperature.

*3.3.2. Chemical Analysis*

The total phenolic content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965) as modified by Gao et al. (2002). Whole beans or seed coats (200 mg) were extracted with acidified methanol (HCl:Methanol:Water, 1:80:10, v/v) (4 mL) at room temperature for 2h on a wrist-action shaker (Burrel, Pittsburgh, PA, USA). The mixture was centrifuged at 1358 x g for 10 min on a table centrifuge (GLC-1, Sorval, Newton, CT, USA) and the supernatant was utilized for determination of the total phenolic content. An aliquot (0.2 mL) was added to 1.5 mL of freshly diluted 10-fold Folin- Ciocalteau reagent (BDH, Toronto, ON, Canada). The mixture was allowed to sit for 5 min and then 1.5 mL of sodium carbonate solution (60 g/L) (Sigma) was added. Afterwards, the mixture was incubated for 90 min and the absorbance read at 725 nm. Acidified methanol was used as a blank and ferulic acid (Sigma) was used as standard. The results were expressed in μg of ferulic acid equivalents per gram of sample.

For measuring the antioxidant activity, 100 mg of finely ground sample (whole beans or seed coats) was extracted in 1 mL of methanol (Fisher) for 2 hours in a rotary shaker. After this period, the samples were centrifuged at 700 x g for 10 min and the supernatant was collected for further analysis. Antioxidant activity was measured using a modified version of Brand-Williams et al. (1995). One hundred μL of the supernatant was reacted with 3.9mL of 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) solution ($6.34 \times 10^{-5}$ M in methanol). The decreasing absorbance was monitored at 515 nm (Ultraspec 200, Pharmacia Biotech Piscataway, NJ) in the dark at 0 and 30 minutes. The reference consisted of 100 μL of methanol in 3.9 mL of DPPH$^{\bullet}$ solution. The results were expressed as a percent of discolouration according with the formula:

$$\left[1-\left(\frac{Absorbance\ Sample_{t=30}}{Absorbance\ Control_{t=0}}\right)\right] X\ 100$$

Phytic acid levels were determined by the method of Latta and Eskin (1980). This analysis was done with a chromatographic column (0.7 cm × 15 cm) containing 0.5 g of an anion-exchange resin (100–200 mesh, chloride form; AG1-X8, Bio-Rad Co.). The process was the same as the AOAC method, and only the digestion step was omitted. The Wade reagent (1 mL, 0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid in distilled water) was added into the extract (3 mL), and centrifuged at 960 x g for 5 min. The absorbance of the supernatant was measured at 500 nm with a UV-VIS spectrophotometer.

### *3.3.3. Physical Analysis*

The raw and the treated samples (whole seeds, cotyledons and seed coats) were analyzed for their moisture (AACC Method 44-01) content using standard methods. Due to various moisture content of samples, all calculations were made according to dry matter basis.

Colour measurements (CIE $L^*$ $a^*$ $b^*$ colour space) were performed using a Minolta CM-3600d model spectrophotometer. Whole bean flours were transferred into a disposable cuvette to measure the reflectance at least twice. The colour of beans was expressed as the average of two $L^*$, $a^*$, and $b^*$ readings, where $L^*$ stands for brightness, $+a^*$ redness, $-a^*$ greenness, $+b^*$ yellowness, and $-b^*$ blueness.

The pasting behavior was measured in a Rapid Visco Analyzer (Model: RVA-4, Newport Scientific Pty. Ltd., Sydney, Australia, 1995) and Thermocline for windows software was used to evaluate the pasting properties. Viscogram profile/pasting curves show the relationships between time, viscosity and temperature during cooking processes. Test runs were conducted following Standard Profile 1 which included: 1 min of mixing, stirring, and warming up to 50 $^{o}$C; 3.7min; of heating at 12 $^{o}$C /min up to 95 $^{o}$C; 2.5 min of holding at 95 $^{o}$C; 3.8min of cooling down to 50 $^{o}$C, at 12 $^{o}$C /min; and 2 min holding at 50 $^{o}$C (Deffenbaugh and Walker, 1989). Starch gelatinization (pasting) curves were recorded by the RVA and viscosity was expressed in terms of centipoises (*cp*).

### *3.3.4. Statistical Analysis*

All data were recorded as means ± SD and analyzed by GraphPad Instat for Windows (ver. 3). One-way analysis of variance (ANOVA), Tukey tests and two-tail t-tests were carried out to test any significant differences between treatments and cultivars. Pearson's correlation coefficient (*r*) was also applied to establish specific correlations. All tests were performed using $\alpha$=0.05.

## 3.4. Results and Discussion

Table 3.1 summarizes the seed coat yield and final moisture contents of different pre-dehulling treatments applied to navy and pinto beans. Increases in the seed coat yield were observed in both navy and pinto beans when these seeds were pre-treated with higher amounts of water. Except when the samples were soaked, tempering followed by HT was more effective in removing the seed coat than the same pre-treatment followed by FD. This may be due to the direct contact of hot air with the seed coats producing a moisture gradient within the seed (i.e., a relatively drier seed surface compared to a relatively high moisture content seed centre). This moisture gradient may have facilitated seed coat removal. The soaking pre-treatment produced the highest seed coat yields for both conditions (FD and HT) for both bean varieties. As expected, the soaking leads to high water absorption and consequent swelling, making the cotyledons expand to break the seed coat integrity, which was then easily detachable due to shrinkage of the cotyledon during drying. In the soaked beans, the use of low or high temperatures did not seem to affect the seed coat yield, even though the final moisture contents varied according to the drying process. Curiously, the seed coat yield of some treatments was higher than the raw samples, which may be explained either by the possibility of micro-fractions of cotyledon contaminating the hull fraction or by the fact that when high amounts of water are applied, the seed coat detaches in its totality, producing heavier particles compared to manual hull removal. Nonetheless, this observation agrees with that of Ehiwe and Reichert (1987) and Anderson et al. (1994).

The total phenolics and the antioxidant activities demonstrated significant variations with respect to bean fraction, bean type and pre-dehulling treatment (Table 3.2). In agreement with the results of Cardador-Martinez et al. (2002) and Rocha-Guzmán et al. (2007), the highest concentration of phenolic compounds occurred in the seed coats.

**Table 3.1.** Seed Coat Yield[a] (%) and Final Moisture Content[b] (%) of Different Pre-dehulling Treatments Applied in Navy and Pinto Beans

| | Seed coat yield | | Final MC (%) | |
|---|---|---|---|---|
| Treatment | Navy beans | Pinto beans | Navy beans | Pinto beans |
| Raw | 14.73 ± 0.54a | 15.24 ± 0.91b,c | 7.86 ± 0.17e* | 8.44 ± 0.22e |
| FD-14 | 14.6 ± 0.37a* | 11.94 ± 0.33a | 5.84 ± 0.08c* | 4.07 ± 0.18b |
| HT-14 | 17.24 ± 0.81b,c | 15.53 ± 0.45b,c | 10.17 ± 0.13f | 10.14 ± 0.15g |
| FD-28 | 15.17 ± 0.74a,b | 13.68 ± 0.67a,b | 4.74 ± 0.16b* | 5.09 ± 0.08c |
| HT-28 | 18.2 ± 0.55c | 16.81 ± 0.78c | 8.19 ± 0.20e* | 9.43 ± 0.23f |
| FD-SK | 20.91 ± 0.43d* | 17.75 ± 0.72c | 2.01 ± 0.06a* | 2.66 ± 0.09a |
| HT-SK | 20.65 ± 0.67d* | 17.38 ± 0.83c | 6.58 ± 0.19d* | 6.96 ± 0.15d |

[a] Results are the means of 3 determinations ± SD adjusted to dry matter.

[b] Moisture content determined in the correspondent whole beans are expressed as the average of 4 determinations.

Means within the same column followed by same character are not statistically significant using one-way ANOVA test ($P< 0.05$).

* Significantly different comparing to pinto beans that received the same treatment using a two-tail t test ($P<0.05$).

The total concentration of phenolics in the seed coats of pinto beans was higher than that observed in navy beans. Seed coat colour of dry beans is determined by the presence and amounts of phenolic compounds, such as flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins) (Feenstra, 1960). This may explain the differences observed in this study; the colour of navy beans seed coats is cream while the seed coat of pinto beans is predominantly brown. Phenolic acid levels were correlated positively with the antioxidant activity evaluated for both navy (whole seeds: $r$=0.9922;

seed coat: $r$=0.9594) and pinto beans (whole seeds: $r$=0.8870; seed coat: $r$=0.9748). Madhujith and Shahidi (2005) and Espinosa-Alonso et al. (2006) suggested that variously-coloured dry beans may be an important source of dietary antioxidants. However, the relationships between colour and phenolic content is controversial. While Barampama and Simard (1993) reported results similar to ours in demonstrating an association between colour and phenol content, Guzmán-Maldornado et al. (1996) did not find similar correlations.

The heating treatments caused a significant increase in the availability of total phenolic that corresponded with an increased antioxidant activity in both whole seeds and seed coats of navy and pinto beans. These changes appear to be related to the time the samples were exposed to high temperature. It was observed that with 14% moisture, antioxidant values for heated seeds averaged 61.4% higher than the FD pre-treatments. At 28% moisture, the difference went to 94.3%. This finding may be explained on the grounds of previous suggestions that some phenolic compounds and their conjugated forms can be converted from one form to another during various technological processes (Stewart et al., 2000; Turkmen et al., 2005). These data indicated that thermal processing of beans under the specified conditions may liberate phenolic compounds and their derivatives from the wall cells. The liberated compounds may then contribute higher antioxidant potential when they are considered as a dietary antioxidant. Fernández et al. (1982) also found a significant increase in phenolic compounds after cooking beans under high pressure.

**Table 3.2.** Total Phenolics (mg ferrulic acid eq/g) and Antioxidant Activity (% of discolouration) of Navy and Pinto Beans (whole seeds and seed coat) Submitted to Different Pre-dehulling Treatments[a]

| | Total phenolics | | Antioxidant activity | |
|---|---|---|---|---|
| Treatment | Whole seeds | Seed coat | Whole seeds | Seed coat |
| Navy | | | | |
| Raw | 0.42 ± 0.04a* | 1.77 ± 0.1a* | 13.82 ± 1.17a* | 35.75 ± 2.71a* |
| FD-14 | 0.47 ± 0.03a* | 1.90 ± 0.09a* | 14.99 ± 0.37a* | 38.86 ± 2.15a* |
| HT-14 | 0.76 ± 0.06b* | 2.1 ± 0.15b* | 19.24 ± 0.88b* | 42.94 ± 2.75a* |
| FD-28 | 0.45 ± 0.01a* | 1.85 ± 0.06a* | 14.91 ± 1.17a* | 39.33 ± 3.35a* |
| HT-28 | 0.85 ± 0.03b* | 2.43 ± 0.13c* | 20.47 ± 1.39b* | 52.48 ± 4.43b* |
| FD-SK | 0.46 ± 0.04a* | 1.72 ± 0.08a* | 15.28 ± 0.66a* | 40.18 ± 2.27a* |
| HT-SK | 0.83 ± 0.08b* | 2.48 ± 0.11c* | 21.05 ± 1.61b* | 54.27 ± 3.59b* |
| Pinto | | | | |
| Raw | 1.92 ± 0.12a | 10.13 ± 0.47a | 43.16 ± 2.59a | 72.34 ± 6.23a,b |
| FD-14 | 1.7 ± 0.10a | 8.94 ± 0.39a | 46.57 ± 1.64a | 70.37 ± 4.31a |
| HT-14 | 2.32 ± 0.22b | 9.31 ± 0.41a | 50.28 ± 3.67b | 69.24 ± 3.38a |
| FD-28 | 2.13 ± 0.1a | 10.68 ± 0.47a | 45.75 ± 2.02a | 72.63 ± 2.61a,b |
| HT-28 | 3.63 ± 0.12c | 12.5 ± 0.72b | 52.31 ± 2.84b | 82.65 ± 4.46b,c |
| FD-SK | 1.89 ± 0.07a | 9.57 ± 0.26a | 44.28 ± 1.39a | 71.05 ± 4.84a |
| HT-SK | 3.61 ± 0.28c | 12.95 ± 0.80b | 53.60 ± 4.23b | 84.46 ±5.46c |

[a] Results are the means of 4 determinations ± SD adjusted to dry matter.

Means within the same column followed by same character are not statistically significant using one-way ANOVA test ($P < 0.05$).

* Significantly different comparing to the same pinto beans fraction (whole seeds or seed coat) that received the same treatment using a two-tail t test ($P<0.05$).

Analyzing the level of bioactive compounds in cereal grains before and after hydrothermal processing, Zieliński et al. (2001) demonstrated an increase of up to 300%

in the content of phenolic acids after extrusion cooking. The antioxidant activity of grape seed extracts has also been shown to be affected by heating conditions, showing a higher reducing power after various heat-treatments (Kim et al., 2006). However, some reports have shown a reduction of total phenolics in beans submitted to different heat treatments (Alonso et al., 2000; Abd El-Hady & Habiba, 2003; Rocha-Guzmán et al., 2007). Although beans are usually consumed cooked and the conditions used in this study had not reached this point, the purpose of this study was to evaluate whether different pre-dehulling treatments can influence the nutraceutical properties of the seed coats and the physical characteristics of the whole seeds. The data show that soaking followed by freeze-drying (FD-SK) does not affect the nutritional parameters displayed in Table 2 (total phenolics and antioxidant activity), which contradicts the findings of Adb El-Hady and Habiba (2003), who suggest that there is a decrease in phenols after soaking due to the leaching of water-soluble phenols into the soaking water. This could be due to the length of the soaking time (16 hrs), which was possibly not effective in reducing the availability of polyphenols in the beans studied.

The phytic acid levels (Table 3.3) in the whole seeds and seed coats of raw navy and pinto beans are in accordance with the findings of Deshpande at al. (1982), who demonstrated a significant increase in this compound after dehulling dry beans ($P$<0.01). These data show the reduction of the phytic acid level from the whole seeds to the seed coats; decreases of over 50% were observed for pinto bean seed coats. While some studies have reported greater phytic acid decreases after soaking and heating (Alonso et al., 2000; Abd El-Hady , 2003; Rehman & Shah, 2005), our data demonstrate no significant difference among the various pre-dehulling treatments. Recently, Hurrell et al.

(2002) reported that high temperatures such as those used in bread making must be applied to degrade phytic acid.

**Table 3.3.** Phytic Acid (mg/g) in Navy and Pinto Beans (whole seeds and seed coat) Submitted to Different Pre-dehulling Treatments[a]

| | Navy beans | | Pinto beans | |
|---|---|---|---|---|
| Treatment | Whole seeds | Seed coat | Whole seeds | Seed coat |
| Raw | 13.5 ± 0.96 | 9.99 ± 0.61* (26.0) | 14.85 ± 0.67 | 6.92 ± 0.22 (53.4) |
| FD-14 | 13.26 ± 0.33 | 9.67 ± 0.48* (27.1) | 14.35 ± 0.85 | 7.02 ± 0.56 (51.1) |
| HT-14 | 13.43 ± 0.47 | 9.85 ± 0.83* (26.7) | 14.42 ± 0.88 | 6.62 ± 0.43 (54.0) |
| FD-28 | 13.1 ± 0.67 | 9.32 ± 0.75* (28.8) | 14.68 ± 0.7 | 6.75 ± 0.37 (53.9) |
| HT-28 | 13.87 ± 0.88 | 10.28 ± 0.7* (25.9) | 13.96 ± 0.78 | 6.94 ± 0.43 (50.3) |
| FD-SK | 13.56 ± 0.42 | 10.21 ± 0.78* (24.7) | 14.2 ± 0.63 | 6.44 ± 0.63 (54.6) |
| HT-SK | 14.09 ± 0.64 | 10.99 ± 0.37* (22.0) | 15.19 ± 0.88 | 7.28 ± 0.85 (52.1) |

[a] Results are the means of 4 determinations ± SD adjusted to dry matter.

Figures in parentheses indicate the percent decrease over the values of the corresponding whole seed.

Means within the same are not statistically significant using one-way ANOVA test ($P$< 0.05).

* Significantly different comparing to the same pinto beans fraction (whole seeds or seed coat) that received the same treatment using a two-tail t test ($P$<0.05).

The physical parameters evaluated showed significant differences under the various treatments studied for both navy and pinto beans (Table 3.4). The difference in the seed coat colours of the bean varieties was confirmed by the variation in the $L^*$, $a^*$ and $b^*$ values of whole raw navy and pinto beans ($P<0.01$), which demonstrated a brighter, greenish, yellow appearance for the navy beans. It can be inferred that the HT caused significant changes in the colour parameters evaluated. The positive $b^*$ values, which indicate yellowness, showed an increasing trend when the exposure time to high temperature changed from 20 to 60 minutes. This observation may be due, at least partially, to the changes in the polyphenolic compounds, ascorbic acid and carotene present in the whole seeds and in the starch fractions, as reported by Galvez and Ressureccion (1993) and Shimelis et al. (2006). Although the major changes were seen in the $b^*$ values (leading to increased yellowness in the HT samples), a decrease in $L^*$ values and an increase in $a^*$ values were also observed under the same conditions.

The pre-dehulling treatments had important effects on the pasting properties. For HT samples, longer and higher exposure to water resulted in lower viscosity values while the FD samples exhibited higher viscosity values. The higher viscosities observed for the FD samples may be due to stronger interactions between starch granules caused by freeze drying, which produced dried seeds with very low moisture contents yet without gelatinizing the starch, therefore affecting the gelatinization process. The high peak viscosities of the FD samples indicate that the amylose granules were probably packed more compactly, reducing the accessibility of water molecules to the binding sites on the amylose chains of the correspondent starches. Thus, the lower the moisture content in the FD beans, the higher were the viscosity values. Alternatively, the HT treatment likely produced a high degree of gelatinization and starch degradation, both of which would result in lower viscosities. Gelatinization of starch has been shown to enhance the extent of hydrolysis in both *in vitro* and *in vivo* studies, and starches in processed foods may be either essentially unchanged, partly or wholly gelatinized, or partly retrograded (Tharanathan & Mahadevamma, 2003). The HT seemed to lead to a high level of retrogradation for the bean starches. Sandhu and Singh (2007) reported a negative correlation between range of retrogradation and peak and final viscosity. Consequently, except for the beverage industry, which could be interested in starches with low final viscosity, the beans submitted to HT would have limited applications in the food industry, as the majority of the existing products demand high viscosity profiles (Su et al., 1997). Prior reports on the effects of heat treatment on the structure and physicochemical properties of cereal and legume starches have suggested that the magnitude of these changes are dependent upon the moisture content during heat treatment and the starch source (Sair, 1967; Lorenz & Kulp, 1981; Hoover & Manuel, 1996).

Nonetheless, it was observed that treated navy and pinto beans that had higher yellowness also presented lower peak viscosities, however this relationship was not statically significant (navy: $r$= -0.6727; pinto: $r$= -0.4768) . Although in general the colour itself does not imply any direct correlation with viscosity, when pre-dehulling treatments were performed, the changes in both colour and viscosity resulted. Moreover, temperature had an important impact on seed coat yield, levels of phenolics, antioxidant activity, colour and pasting properties, causing no effect on phytic acid levels.

Navy and pinto beans, raw or treated, showed different profiles. Cultivar had a significant role ($P$<0.05) on the seed coat yield and nutritional and physical properties. While navy beans yielded higher seed coat amounts after the soaking treatments and presented higher levels of phytic acid in the seed coats, pinto beans demonstrated higher phenolics concentration and antioxidant activity, besides showing higher viscosity values after the applied conditions.

**Table 3.4.** Colour Analysis and Pasting Properties of Whole Navy and Pinto Bean Flours Submitted to Different Dehulling Treatments[a]

| | Colour Analysis[b] | | | Pasting Properties (*cp*) | |
|---|---|---|---|---|---|
| Treatment | *L** | *a** | *b** | Peak Viscosity | Final Viscosity |
| Navy | | | | | |
| Raw | 86.88 ± 0.09b* | -0.24 ± 0.01b,c* | 9.38 ± 0.24b* | 154.5 ± 4.95c | 401.0 ± 21.21c |
| FD-14 | 86.29 ± 0.18b* | -0.09 ± 0.01d* | 9.52 ± 0.02b* | 95.0 ± 7.07b* | 251.5 ± 2.12b |
| HT-14 | 85.55 ± 0.10a* | -0.19 ± 0.01c* | 11.48 ± 0.08c* | 56.0 ± 15.56a | 73.0 ± 11.31a* |
| FD-28 | 87.56 ± 0.01b* | -0.25 ± 0.01b* | 9.32 ± 0.10b | 188.0 ± 7.78d* | 463.0 ± 18.39c* |
| HT-28 | 84.87 ± 0.09a* | 0.04 ± 0.00e* | 13.74 ± 0.03d* | 54.0 ± 12.73a | 69.5 ± 13.44a* |
| FD-SK | 89.43 ± 0.50c* | -0.46 ± 0.02a* | 8.78 ± 0.03a* | 476.0 ± 19.80e | 1124.5 ± 21.92d* |
| HT-SK | 85.12 ± 0.12a* | 0.12 ± 0.02f* | 14.40 ± 0.12e* | 31.0 ± 2.83a | 24.5 ± 4.95a |
| | | | | | |
| Pinto beans | | | | | |
| Raw | 82.72 ± 0.05d | 1.10 ± 0.01c | 8.55 ± 0.01a | 152.5 ± 19.09d | 334.5 ± 53.03b |
| FD-14 | 83.56 ± 0.10e | 1.04 ± 0.04c | 8.98 ± 0.03b | 130.0 ± 8.49c,d | 300.5 ± 19.09b |
| HT-14 | 82.13 ± 0.03c | 1.30 ± 0.02d | 9.44 ± 0.09c | 103.0±18.39a,b,c | 117.5 ± 4.95a |
| FD-28 | 84.28 ± 0.04f | 0.89 ± 0.03b | 9.06 ± 0.02b | 271.0 ± 19.80e | 594.5 ± 30.41c |
| HT-28 | 80.87 ± 0.08b | 1.57 ± 0.01e | 10.99 ± 0.12d | 62.0 ± 7.07a,b | 136.5 ± 16.26a |
| FD-SK | 85.88 ± 0.22g | 0.60 ± 0.02a | 9.12 ± 0.06b | 547.5 ± 37.48f | 1331.5 ± 0.71d |
| HT-SK | 79.64 ± 0.20a | 1.46 ± 0.06e | 13.13 ± 0.03e | 47.0 ± 11.31a | 43.0 ± 11.31a |

[a] Results are the means of 3 determinations ± SD.

[b] *L**- lightness(black/white), *a**- chroma (green/red) and *b**- hue (blue/yellow).

Means within the same column followed by same character are not statistically significant using one-way ANOVA test ($P < 0.05$).

* Significantly different comparing to pinto beans that received the same treatment using a two-tail t test ($P<0.05$).

These findings indicate that, depending on the temperature and moisture conditions, important nutritional and physical properties of beans can be manipulated to yield end products with specific characteristics. The impact of the various pre-dehulling treatments on nutraceutical value and corresponding characteristics of the whole seeds demonstrated that research must be continued in this area, to achieve feasible processes for the preparation of high quality products.

## 4. Influence of Added Bean Flour (*Phaseolus vulgaris* L.) on Some Physical and Nutritional Properties of Wheat Flour Tortillas

### 4.1. Abstract

Composite flours containing 15, 25, or 35% of small red, black, pinto, or navy bean flours (BF) and wheat were made into tortillas. Dough rheology, firmness, cohesiveness, rollability, and some physical properties of tortillas were negatively affected as BF concentration increased regardless of bean cultivar. Nutritionally, all bean tortillas had significantly higher levels of crude protein, total phenols, 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) *in vitro* antioxidant activity (AA) and antinutritional compounds such as phytic acid (PA) and trypsin inhibitors (TI) than the wheat control. Tortillas to which 35% of small red, pinto and black BF was added had the highest levels of phenols, which were significantly correlated with both $DPPH^{\bullet}$ ($r$=0.99) and $ABTS^{\bullet+}$ ($r$= 0.99) AA. Compared to raw flours, PA and TI were reduced from 37.37 to 43.78% and from 50 to 66%, respectively, in the tortillas. Overall analysis indicated that tortillas with acceptable texture and improved nutritional profile were produced at 25% substitution.

### 4.2. Introduction

Flour tortillas are a unique baked product that have been produced in Mexico for centuries. The major ingredient for production of flour tortillas is wheat flour and the final product can be defined as a flat, circular, light-coloured bread. Tortillas are generally eaten with beans, meats, cheese, avocados, spreads, and other ingredients (Waniska, 1999).

Tortillas are now more popular in United States than bagels, croissants, English muffin, pitas, or any other type of ethnic bread. In 2005, the baking industry in United States showed a consistent increase in the flour tortilla market. While the consumption of fresh bread was up 0.3%, the increase in tortilla sales was up 3.5% in comparison to the previous year (Kuk, 2006). The reconstruction of the tortilla with new nutritional attributes and new formats has been part of this growth (Berne, 2005).

Nutritionally, wheat based flour tortillas are rich in carbohydrates that generate a high glycemic response after ingestion, similar to white bread (Saldana & Brown, 1984). On the other hand, common bean (*Phaseolus vulgaris* L.) is low in fat and rich in proteins, vitamins, complex carbohydrates and minerals. In addition to contributing nutritional requirements, consumption of dry beans has been linked to reduced risk of heart disease (Anderson et al., 1984; Winham & Hutchins, 2007), obesity (Geil & Anderson, 1994) and cancer (Garcia-Gasca et al., 2002; Azevedo et al., 2003).

Economically, beans are an important crop in North America, since their production and export has increased significantly in recent years (Agriculture and Agri-Food Canada, 2005). However, widespread use of beans as a primary staple food has been limited by the presence of antinutritional factors, which might produce adverse effects for human and animal nutrition. Some of these compounds include phytic acid and trypsin inhibitors (Martin-Cabrejas et al., 2004). While trypsin inhibitors have been reported to be thermolabile and therefore easily inactivated during thermal processing, phytic acid is known to be quite stable in thermal treatments, undergoing only partial hydrolysis (Abd El-Hady & Habiba, 2003; Estévez, Castillo et al., 2005).

Nonetheless, consumption of coloured beans may play an important role against oxidative stress due to the presence of polyphenols that possess *in vitro* antioxidant activity (Beninger & Hosfield, 2003; Madhujith & Shahidi, 2005; Anton et al., 2008b). It is believed that antioxidants scavenge free radicals and reactive oxygen species and can play an important role in inhibiting oxidative mechanisms that lead to degenerative diseases (Madhujith & Shahidi, 2005). These compounds have been promoted by health authorities, thus stimulating the consumption of foods rich in antioxidants (USDA, 2005). Increasing the supply of antioxidants in staple foods such as bread and tortillas may provide a safety net for those who cannot or do not want to consume fruit and vegetables. As a result of this trend, attempts to fortify commonly consumed white bread with antioxidants have been reported (Park et al., 1997a; Park et al., 1997b).

Because of their nutritional and health promoting properties, the development of value-added bean-based products for new market opportunities in the functional food and nutraceutical industry is being promoted (Singh, 1999). Attempts aimed to improve the nutritional profile of bread by adding hulls and cotyledons of legumes have been reported (Doxastakis et al., 2002; Dalgetty & Baik, 2006), however few publications have focused on improving the nutritional profile of wheat based flour tortillas (Gonzalez-Agramon & Serna-Saldivar, 1988; Friend et al., 1992; Serna-Saldivar et al., 2004).

The purpose of the current study was to examine the effect of adding flours from different bean cultivars at varied levels on some physical and nutritional properties of wheat based flour tortillas. Physically, dough rheology and characteristics of the final product such as firmness, cohesiviness, colour, diameter, thickness, and rollability were investigated. Protein content, total phenolics, antioxidant activity, and levels of phytic acid and trypsin inhibitors were determined in order to verify the nutritional changes.

## 4.3. Materials and Methods

### *4.3.1. General*

Small red (AE: AC Earlired), black (BV: Black Violet), pinto (AP: AC Pintoba), and navy (GTS: GTS 531) beans were obtained from the Agriculture and Agrifood Canada Research Station in Morden, MB, Canada. The cultivars were grown and harvested in 2005 and exposed to the same environmental conditions in order to avoid external variation. The weight of 100 seeds was determined gravimetrically and expressed as mean ± SD of 3 determinations. Crude protein content of bean samples were: 22.26% for AE, 25.29% for BV, 25.61% for AP, and 26.42% for GTS.

Whole seeds were ground in a Jacobson pilot scale hammer mill (Model No 120-B, Minneapolis, MN, USA) to pass a 500 µm sieve (35 mesh US Standard Sieve Series). Ground samples were added at different levels (15, 25, and 35%) to Canadian hard red spring wheat (Laura variety, crop year 2005; 13.55% crude protein) flour and the composite flours were stored at 5 °C in opaque, closed containers for further use.

### *4.3.2. Dough Rheology*

The effect of adding bean flour on dough mixing properties was determined using a Brabender Farinograph (Diusburg, Germany) according to the constant flour weight procedure (Method 54-21; AACC, 1995). The parameters determined were % water absorption, dough development time (DDT), and dough stability.

### *4.3.3. Tortillas Preparation*

100g of flour (either wheat or composites) with weight adjusted to 14% of moisture content was added to 9 g of vegetable shortening (Crisco, Markham, ON, Canada), 1.5g of baking powder (Magic Baking Powder, Kraft Canada Inc., Don Mills, ON, Canada), 1.5g of sodium chloride (Food Grade, Fisher, Ottawa, ON, Canada) and distilled water. The amount of added water was determined based on the Farinograph Water Absorption (FAB) and previous tests that determined the optimum water addition for making tortillas with the best cohesiviness possible (results not shown). The tests indicated that the final product would achieve its best by adding the FAB value minus 12 g of water. The dry ingredients plus shortening were placed onto a 200g mixer (National MFG. Co, Lincoln, NB, USA) and mixed for 2 minutes before water addition. Once water was added, the batter was further mixed for 2 min (35% composites), 3 min (25% composites), or 4 minutes (15% composites and wheat flour). Mixing time was determined by previous tests that identified the average mixograph peak stability time for each level of substitution (results not shown). After mixing, dough was cut into pieces of

35g. These pieces were rounded, pinched on the bottom and placed in separate plastic containers, covered with a damp cloth and allowed to rest for 5 minutes at room temperature. After resting, each 35g ball was slightly flattened by hand and pressed on a hot press (Doughpro Proprocess Corporation, Paramount, CA, USA) previously heated to 93 °C (top and bottom platens) for 8s. Thereafter, tortillas were transferred to an electric hot plate preheated at 218 °C and baked for 30s on the first side, flipped and baked for 40s on the second side, and flipped and baked for another 10s on first side. Baked tortillas were cooled on a rack for 1 min and packed in open polyethylene plastic bags. The bags were sealed after 3 h and left at 25 °C overnight. Physical properties of tortillas were evaluated 24 h after production. Each batch yielded 4 tortillas. Chemical analysis and moisture of tortillas were determined after tortillas were dried at −50 °C, 5 Pa, for 48 h in a freeze dryer (Genesis 25 & 35 Freeze Dryer, SP Industries, Warminster, PA, USA) with samples previously frozen for 16 h at -40 °C.

The raw composite and wheat flours, as well as the freeze-dried tortillas, were analyzed for their moisture content by AOAC method 925.10 (AOAC, 1990). Due to the different moisture content of samples, all calculations were made on a dry matter basis.

### *4.3.4. Physical Analysis*

Tortilla firmness was determined by a puncture test with a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) equipped with a cylindrical probe (TA 108, 18 mm diameter) with a force of 40g. Tortillas were placed blistered side down on a tortilla burst rig (in accordance with the manufacturer's instructions) and firmness was measured as the resistance to puncture (peak force). Cohesiveness was calculated as the work during compression (rupture force multiplied by the distance at peak force).

Diameter was measured with a ruler and thickness with a caliper at three different places in each tortilla and the mean was calculated for each (one value was considered for each tortilla). Rollability was evaluated by wrapping a tortilla around a dowel (1.0 cm diameter) and rating the cracking and breakage of the tortilla. Rollability was rated in a scale of 1-6, where 1=no signs no cracking (best), 2=edge cracking only, 3=edge cracking and/or cracking in the center, 4=cracking and breaking on one side, 5=cracking and breaking on both sides (clean break) but still rollable, 6=unrollable.

Colour measurements (CIE $L^*$ $a^*$ $b^*$ colour space) were performed using a Minolta CM-3600d model spectrophotometer. The colour of tortillas was expressed as the average of two $L^*$, $a^*$, and $b^*$ readings, where $L^*$ stands for brightness, $+a^*$ redness, $-a^*$ greenness, $+b^*$ yellowness, and $-b^*$ blueness. A white calibration plate was used to standardize the equipment prior to colour measurements.

### *4.3.5. Chemical Analysis*

Nitrogen content was determined by using the Kjeldahl method and was multiplied by a factor of 5.7 to estimate protein content (AOAC, 1990).

For determination of total phenol content and antioxidant activity, 100 mg of finely ground sample was extracted in 2.5 mL of acetone/water (80:20, v/v) (Fisher, Ottawa ON) for 2 h in a rotary shaker. After this period, the samples were centrifuged at

3,000 x g in a table centrifuge (GLC-1, Sorval, Newton, CT, USA) for 10 min..Thereafter the supernatant was transferred to a 3 ml syringe (Fisher) and filtered through a 0.45 μm sterile PVDF filter unit (Fisher). The filtrate was collected for further analysis. Preliminary tests on extraction solvents revealed that methanol was unsuitable for extraction of phenolic compounds under such conditions, producing extracts with high degree of turbidity and unacceptable standard deviation in these tests.

The total phenolic content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965) as modified by Gao et al. (2002). An aliquot (0.2 mL) of extract was added to 1.5 mL of freshly diluted 10-fold Folin- Ciocalteau reagent (BDH, Toronto, ON, Canada). The mixture was allowed to sit for 5 min and then 1.5 mL of sodium carbonate solution (60 g/L) (Sigma, St Louis, MO, USA) was added. Afterwards, the mixture was incubated for 90 min and the absorbance read at 725 nm. Acetone/water (80:20, v/v) was used as a blank and ferulic acid (Sigma, St Louis MO) was used as standard. The results were expressed in mg of ferulic acid equivalents per 100 g of sample. Linearity range of the calibration curve was 20 to 200 μg ($r$=0.99).

For measuring the antioxidant activity two methods were employed. Antioxidant activity was initially measured using a modified version of Chen and Ho (1995). For this assay, 200 μL of extract was reacted with 3.8mL of 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) solution ($6.34 \times 10^{-5}$ M in methanol). The decreasing absorbance was monitored at 517 nm (Ultraspec 200, Pharmacia Biotech Piscataway, NJ) in the dark at 30 minutes against methanol blank. The control consisted of 200 μL of acetone/water (80:20, v/v) in 3.8 mL of $DPPH^{\bullet}$ solution. The results were obtained as a percent of discolouration according with the formula:

$$\left[1-\left(\frac{Absorbance\ Sample}{Absorbance\ Control}\right)\right]X\ 100$$

Simultaneously to the samples, 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) (Sigma) was used as a standard and the results were expressed as μmol of Trolox equivalents per 100 g of sample. Linearity range of the calibration curve was 0.25 to 2.0 μmol ($r$=0.99).

The second method for determining antioxidant activity was the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$) radical cation decolourization assay (Re et al., 1999). The ABTS radical cation ($ABTS^{+\bullet}$) was produced by reacting 7 mM ABTS (Sigma) stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 16 h before use. The $ABTS^{+\bullet}$ solution (2 days stability) was diluted with methanol to an absorbance of 0.70 ± 0.02 at 658 nm. After addition of 100 μl of extract or Trolox standard to 2.9 mL of diluted $ABTS^{+\bullet}$ solution, tubes were incubated for 15 min at 30°C and absorbance was measured at 734 nm. Solutions of known Trolox concentrations in acetone/water (80:20, v/v) were used for calibration. Linearity range of the calibration curve was 0.25 to 2.0 μmol ($r$=0.99).

Phytic acid levels were determined in flours and tortillas by the method of Latta and Eskin (1980). This analysis was done with a chromatographic column (0.7 cm × 15 cm) containing 0.5 g of an anion-exchange resin (100–200 mesh, chloride form; AG1-X8, Bio-Rad Co.). The process was the same as the AOAC method, and only the digestion step was omitted. The Wade reagent (1 mL, 0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid in distilled water) was added into the extract (3 mL), and the mixture vortexed for 30s. The absorbance of the supernatant was measured at 500 nm with a UV-VIS spectrophotometer.

Trypsin inhibitor activity was measured following the procedure by Kakade et al. (1974), using α-*N*-benzoyl-DL-arginine-*p*-nitroanilidehydrochloride (BAPNA) (Sigma) as the substrate for trypsin. 500 mg of finely ground sample was extracted with 25 mL of 0.01N sodium hydroxide for 3 hours at room temperature in a rotatory shaker. Extracts were centrifuged at 17,500 x g (RC5C, Sorval, Newton, CT, USA) at 4°C for 20 min, and the supernatants filtered through Nb 1 Whatman filter paper. Thereafter extracts were diluted to 30% in distilled water so that 1.0 mL could inhibit 50% of trypsin activity in the conditions presented herein. Five portions of extracts (0, 0.6, 1.0, 1.4, and 1.8 mL) were pipetted into test tubes and the final volume was adjusted to 2 mL with distilled water. Trypsin solution (2 mL, 20 mg/L in 0.001M HCl) was added and the tubes were placed in the water bath at 37°C, followed by addition of 5 mL of BAPNA solution (0.4 mg/mL in Tris-buffer 0.05 M, pH 8.2) previously warmed to 37°C. After exactly 10 min the reaction was stopped by adding 1 mL of 30% acetic acid to each test tube. The absorbance was read at 410 nm and the reagent blank prepared by adding 1 mL of 30% acetic acid to a test tube containing trypsin and water (2 mL of each) before the BAPNA solution was added. One trypsin unit was arbitrarily defined as an increase of 0.01 absorbance unit at 410 nm per 10 mL of the reaction mixture under the conditions used herein.

### *4.3.6. Statistical Analysis*

All data were recorded as means ± SD and analyzed by GraphPad Instat for Windows (ver. 3). One-way analysis of variance (ANOVA), Tukey tests and two-tail t-tests were carried out to test any significant differences between treatments and cultivars. Pearson's correlation coefficient (*r*) was also applied to establish specific correlations. All tests were performed using $\alpha$=0.05.

## 4.4. Results and Discussion

### *4.4.1. Effect of Added Bean Flour on Dough Rheology*

The effect of bean flour addition on tortilla dough rheology is summarized in Table 4.1. Water absorption was increased by addition of bean flour as a function of increased rate of substitution. Substituting wheat flour with 15% bean flour resulted in increases in water absorption of 3 to 5%. This effect was more pronounced in 35% substitutions, where the increase in water absorption was up to 8%. Since most bean proteins are water soluble (Deshpande et al., 1983; Morales-de-Léon et al., 2007) and gluten, the most relevant wheat protein, is mostly water insoluble, the higher water absorption of the composites could be related to the high water absorption of the beans (Deshpande et al., 1983). The time required for dough development or time necessary to reach 500 BU of dough consistency (DDT) clearly decreased as bean concentration increased. During this phase of mixing, the water hydrates the flour components and the dough is developed. Dough-mixing studies showed that inclusion of bean flour highly impacted the time the dough maintains its best consistency at the 500 BU line. Dough stability decreased abruptly as bean flour was added. This is logical since beans are absent of proteins that give wheat dough its viscoelastic properties (Deshpande et al., 1983). Therefore, as the concentration of wheat flour, and consequently its gluten content, is decreased by the addition of bean flour the dough rheological properties are negatively affected.

**Table 4.1.** Effect of Added Bean Flours on Tortillas Dough Rheology Properties

| Flour | Concentration (%) | WA (%) | DDT (min) | Stability (min) |
|---|---|---|---|---|
| Wheat | 100 | 65 ± 0.1 | 8.7 ± 0.2 | 13.7 ± 0.1 |
| AE | 15 | 68.3 ± 0.2a* | 8.5 ± 0.5a | 6.8 ± 0.25c* |
| | 25 | 68.85 ± 0.25b* | 8.05 ± 0.35a | 4.6 ± 0.1b* |
| | 35 | 69.95 ± 0.25c* | 7.85 ± 0.35a | 2.7 ± 0.06a* |
| BV | 15 | 68.3 ± 0.13a* | 6.8 ± 0.35a* | 5.9 ± 0.1c* |
| | 25 | 69.2 ± 0.25b* | 6.7 ± 0.25a* | 3.7 ± 0.1b* |
| | 35 | 69.8 ± 0.1c* | 6.85 ± 0.15a* | 2.1 ± 0.1a* |
| AP | 15 | 67.5 ± 0.58a* | 8.40 ± 0.17a | 5.93 ± 0.06c* |
| | 25 | 67.7 ± 0.17a* | 8.27 ± 0.40a | 4.33 ± 0.29b* |
| | 35 | 67.76 ± 0.12a* | 9.27 ± 0.23b | 3.63 ± 0.31a* |
| GTS | 15 | 68.83 ± 0.46a* | 7.1 ± 0.17a* | 5.27 ± 0.12c* |
| | 25 | 69.2 ± 0.52a,b* | 6.67 ± 0.29a* | 2.73 ± 0.06b* |
| | 35 | 70.1 ± 0.4b* | 7.2 ± 0.25a* | 1.8 ± 0.15a* |

WA: water absorption; DDT: dough development time.

AE: small read bean variety AC Earlired; BV: black bean variety Black Violet; AP: pinto bean variety AC Pintoba; GTS: navy bean variety GTS 531.

All the values are Mean ± SD of three determinations. Data followed by the same character in the same column, within the same bean flour, are not significantly different ($P>0.05$).

*Significantly different comparing to wheat control using a two-tail t-test ($P<0.05$).

Dough from black (BV) and navy (GTS) bean composite flours demonstrated higher water absorption, and lower DDT and stability. This was probably due to the higher amount of fibre from the seed coats in these flours, which is based on the weight of 100 seeds (AE: 24.46 ± 2.65g; BV: 17.74 ± 1.65g; AP: 42.83 ± 1.75g; GTS: 15.97 ± 0.29g); when the mass of the seeds is less, the seed coat comprises a larger area relative to one whole seed. Such an effect has been reported by Dalgetty and Baik (2006), who discussed the addition of hulls from legumes on dough rheology.

#### *4.4.2. Effect of Added Bean Flour on Some Physical Properties of Tortillas*

The effect of added bean flour on some physical characteristics of flour tortillas is shown on Table 4.2. Addition of bean flour, regardless of cultivar or concentration, significantly affected firmness and cohesiveness of tortillas. As observed with the dough from composite flours, firmness and cohesiveness decreased as a function of increased substitution of wheat with bean flour. Firmness was reduced from 23.8 to 28.73%, in flours containing 15% bean flour, 37.32 to 43.66% for 25% substitutions, and 52.02 to 62.15% for 35% substitutions. Although the effect of level of substitution was significant for all bean cultivars, the effect of bean cultivar on firmness was significant only in tortillas to which 35% bean flour was added (Table 4.3). Cohesiveness, which accounts for the time and force necessary for rupture of tortilla, showed more visible differences amongst all the levels of substitutions and cultivars. This parameter was decreased by

53.37 to 59.05% in 15% bean tortillas, 63.81 to 73.11% in 25% substitutions, and 77 to 86.37% in 35% substitutions. The effect of cultivar was significant when tortillas contained 25% and 35% bean flour. In general, small red and navy beans (AE and GTS) composite flours were shown to produce tortillas with the best firmness and cohesiveness, however these parameters were not correlated with dough rheological properties. This negative impact on textural parameters due to addition of increasing levels of alternative ingredients on wheat tortilla formulations have been reported for oat bran and rice bran (Friend et al., 1992) and triticale (Serna-Saldivar et al., 2004). Physical properties such as diameter showed an increasing trend as the level of substitution increased, whereas thickness showed the opposite, indicating thinner tortillas at higher bean flour concentrations. Dalgetty and Baik (2006) showed a reduction in volume of bread resulted from increased levels of substitution of wheat with legume fibres. In the case of tortillas, as puffiness is decreased, demonstrated by decreased thickness, diameter is increased. Factors such as those described for the negative dough properties of bean composite flours may justify the changes in diameter and thickness. Furthermore, it is hypothesized that bean flour addition is greatly affecting the gluten network of flour tortillas.

Tortillas containing 35% of bean flour were less rollable than those added of 0, 15, or 25% bean flour regardless of cultivar, i.e., tortillas broke more easily during rollability test.

**Table 4.2.** Effect of Added Bean Flours on Some Physical Properties of Wheat Tortillas

| Flour | % | Firmness (g) | Cohesiveness (g/s) | Diameter (cm) | Thickness | Rollability | $L^*$ | $a^*$ | $b^*$ |
|---|---|---|---|---|---|---|---|---|---|
| Wheat | 100 | 993.44 ± 40.81 | 10185.57 ± 116.95 | 16.02 ± 0.62 | 1.33 ± 0.25 | 1 | 81.87 ± 0.43 | -0.85 ± 0.2 | 23.65 ± 3.48 |
| AE | 15 | 757.11 ± 14.93c* | 4749.26 ± 82.72c* | 16.4 ± 0.32a | 1.6 ± 0.11b | 1a | 74.58 ± 1.22c* | 3.8 ± 0.42a* | 18.35 ± 0.72b* |
| | 25 | 622.73 ± 18.53b* | 3680.46 ± 126.63b* | 16.55 ± 0.48a | 1.4 ± 0.15a,b | 1a | 72.58 ± 0.54b* | 4.27 ± 0.33a* | 16.85 ± 0.29a* |
| | 35 | 480.02 ± 5.3a* | 2280.86 ± 26.97a* | 16.62 ± 0.38a | 1.28 ± 0.17a | 2b* | 68.13 ± 1.13a* | 5.46 ± 0.38b* | 15.8 ± 0.61a* |
| BV | 15 | 733.57 ± 6.29c* | 4602.63 ± 52.1c* | 16.63 ± 0.08a | 1.28 ± 0.15a | 1a | 68.89 ± 1.4b* | -0.26 ± 0.12a | 11.16 ± 0.51b* |
| | 25 | 559.67 ± 7.25b* | 2738.55 ± 71.72b* | 17.18 ± 0.15b* | 1.07 ± 0.1a | 1a | 59.99 ± 4.61a* | -0.1 ± 0.34a,b* | 8.29 ± 0.76a* |
| | 35 | 413.8 ± 20.41a* | 1586.81 ± 134.19a* | 17.43 ± 0.15b* | 1.07 ± 0.12a | 2.5 ± 0.71b* | 57.45 ± 1.77a* | 0.29 ± 0.26b* | 7.43 ± 0.93a* |
| AP | 15 | 708 ± 38.18c* | 4171 ± 222.03c* | 16.27 ± 0.15a | 1.55 ± 0.08b | 1a | 76.82 ± 0.93c* | 2.36 ± 0.46a* | 21.17 ± 0.89a |
| | 25 | 568.5 ± 9.19b* | 2846 ± 61.22b* | 16.9 ± 0.29b | 1.27 ± 0.15a | 1a | 73.84 ± 0.75b* | 3.04 ± 0.16b* | 20.8 ± 0.32a |
| | 35 | 376 ± 11.31a* | 1388.5 ± 2.12a* | 17.05 ± 0.23b* | 1.13 ± 0.12a | 2b* | 71 ± 0.42a* | 3.76 ± 0.19c* | 20.25 ± 0.46a |
| GTS | 15 | 748.22 ± 21.77c* | 4493.48 ± 68.98c* | 16.7 ± 0.39a | 1.25 ± 0.18a | 1a | 82.87 ± 0.49a | -0.62 ± 0.16a | 22.63 ± 1.21a |
| | 25 | 588.57 ± 18.36b* | 2896.99 ± 97.59b* | 16.93 ± 0.34a* | 1.13 ± 0.12a | 1.5 ± 0.71a,b* | 82.69 ± 0.58a | -0.78 ± 0.15a | 24.54 ± 1.95a,b |
| | 35 | 516.8 ± 27.87a* | 2337.64 ± 85.75a* | 17.2 ± 0.1a* | 1.11 ± 0.1a | 2.5 ± 0.71b* | 82.44 ± 0.36a | -0.8 ± 0.16a | 26.13 ± 1.31b |

$L^*$: black/white; $a^*$: green/red; $b^*$: blue/yellow.

AE: small read bean variety AC Earlired; BV: black bean variety Black Violet; AP: pinto bean variety AC Pintoba; GTS: navy bean variety GTS 531.

All the values are Mean ± SD of four determinations. Data followed by the same character in the same column, within the same bean flour, are not significantly different ($P$>0.05).

*Significantly different comparing to wheat control using a two-tail t-test ($P$<0.05).

Except for tortillas to which navy bean flour was added, colour was highly affected by incorporating small red, black, or pinto beans in flour tortilla formulations. Black bean flour addition caused the highest change in colour in comparison to the wheat control. Although different than the control, small red and pinto bean flour tortillas did not differ significantly between each other in regards to lightness ($P>0.05$).

**Table 4.3.** Effect of Bean Cultivar on Some Physical and Nutritional Properties of Flour Tortillas

| | Bean Flour Concentration (%) | | |
|---|---|---|---|
| | 15 | 25 | 35 |
| WA | * | ** | *** |
| DDT | *** | *** | *** |
| Stability | *** | *** | *** |
| Firmness | NS | NS | *** |
| Cohesiviness | NS | * | *** |
| Diameter | NS | NS | ** |
| Thickness | ** | * | NS |
| Rollability | NS | NS | NS |
| $L^*$ | *** | *** | *** |
| $a^*$ | *** | *** | *** |
| $b^*$ | *** | *** | *** |
| Protein | * | ** | * |
| TP | *** | *** | *** |
| $AOX^1$ | *** | *** | *** |
| $AOX^2$ | *** | *** | *** |
| PA Raw Flours | * | ** | ** |
| PA Tortillas | NS | *** | *** |
| TI Raw Flours | *** | *** | *** |
| TI Tortillas | *** | *** | *** |

WA: water absorption; DDT: dough development time; $L^*$: black/white; $a^*$: green/red; $b^*$: blue/yellow; TP: total phenol content; $AOX^1$: $DPPH^\bullet$ antioxidant activity; $AOX^2$: $ABTS^{+\bullet}$ antioxidant activity; PA: phytic acid; TI: trypsin inhibitors.

NS: no significant effect ($P>0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

### *4.4.3. Effect of Added Bean Flour on Some Nutritional Properties of Tortillas*

Changes in selected nutritional properties of tortillas are summarized in Table 4.4. The addition of bean flour to wheat flour was expected to increase the protein content of the final product, since legumes generally contain more proteins than cereals (Tharanathan & Mahadevamma, 2003). In fact, even at the lowest concentration bean tortillas were 13.6% on average richer in protein than the control. Significant increases were observed at all levels of substitution; however, 25% and 35% substitutions resulted in more evident changes. More relevant than that is the consequent enhancement on the amino acid profile of wheat tortillas. Although amino acids were not evaluated in this study, the literature shows that addition of legume flour on wheat flour baked products improves the essential amino acid balance of such foods (Koehler et al., 1987; Shehata et al., 1988; Tharanathan & Mahadevamma, 2003).

Levels of total phenols and antioxidant activities demonstrated significant variations with respect to bean flour concentration and bean cultivar. Tortillas made out of composite flours containing coloured dry beans (AE, BV, and AP) had significantly higher ($P<0.001$) levels of total phenols and antioxidant activity than those formulated with navy bean flour (GTS) or wheat flour alone; yet navy bean flour tortillas were significantly higher in these parameters than the control, the difference was remarkably higher when comparing navy bean and wheat flour tortillas to those added of coloured beans. The colour of dry beans is determined by the concentration of phenolic compounds, such as flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins) in the seed coat (Feenstra, 1960). This may explain the differences observed in this study as the colour of navy beans seed coats is cream white, whereas the seed coats of the other cultivars are dark red (AE), black (BV) or predominantly brown (AP). In all tortillas levels of total phenols were significantly ($P$<0.01) correlated with both DPPH$^{\bullet}$ ($r$= 0.975) and ABTS$^{+\bullet}$ ($r$= 0.956) antioxidant activities. Antioxidant activity determined by the ABTS$^{+\bullet}$ radical (Re et al., 1999) was to a great extent higher than the results from the DPPH$^{\bullet}$ radical assay. Similar observations have however been described by Sánchez et al. (2007) and Wang and Ballington (2007). Also, our findings are in accordance with Madhujith and Shahidi (2005) and Espinosa-Alonso et al. (2006), who suggested that while coloured dry beans may be an important source of dietary antioxidants, the method of determination of antioxidant activity plays an important role in the quantification of antioxidant capacity of these foods. As expected, tortillas containing the highest concentration of small red (AE), black (BV), or pinto (AP) bean flours showed the highest antioxidant activities regardless of antioxidant assay. The effect of bean cultivar was clearly visible and significant at all levels of substitution (Table 3). Analysis of variance showed that among coloured bean tortillas, small red had significant ($P$<0.05) higher levels of total phenols and antioxidant activities (both DPPH$^{\bullet}$ and ABTS$^{+\bullet}$) in all levels of substitution, followed by pinto and black bean tortillas.

The presence of antinutritional compounds has limited the use of bean for human and animal consumption. The phytic acid contents of the bean tortillas varied from 18.15 to 43.97 mg/10g (Table 4.5). Tortillas containing higher levels of bean flour, and consequently higher protein content, had higher phytic acid levels. This compound has been reported to be quite stable in thermal treatments, undergoing only partial hydrolysis (Estévez et al., 1991; Abd El-Hady & Habiba, 2003; Rehman & Shah, 2005).

Nonetheless, our data shows that a consistent reduction in phytic acid levels occurred in composite flours processed into tortillas.

Phytic acid levels were reduced to levels ranging from 37.37 to 43.78% in flours subjected to the type of heat treatment usually employed in hot-press flour tortilla production (considering only the composite flour portion of tortilla formulation, equal to 90%; i.e., excluding shortening, salt and baking powder). Such reductions may be explained on the basis that during cooking inositol hexaphosphate could have been hydrolyzed to lower molecular weight forms, which is in agreement with the work of Alonso et al. (2000), who reported a significant reduction in phytic acid content in beans submitted to extrusion cooking. Similar reductions in phytic acid levels in whole beans processed under various conditions, including soaking, roasting, autoclaving, and pressure-cooking have been reported (Alonso et al., 2000; ElMaki et al., 2007; Shimelis & Rakshit, 2007), however a direct comparison with these studies is difficult since the biochemical reactions involved in the processing of whole seeds and flour of legumes occur in a different manner (Alonso et al., 2000).

Of the protease inhibitors present in legumes, the most important are the trypsin inhibitors, whose action has been thoroughly studied (Deshpande et al., 1983; Estévez et al., 1991; Adb El-Hady et al., 2003). Navy bean composite flours and tortillas showed the highest levels of trypsin inhibitors, while small red had the lowest (Table 4.5).

**Table 4.4.** Effect of Added Bean Flours on Some Nutritional Properties of Wheat Tortillas

| Flour | % | Protein (g/100g) | TP (mg FAE/100g) | AOX[1] (μmol TE/100g) | AOX[2] (μmol TE/100g) |
|---|---|---|---|---|---|
| Wheat | 100 | 10.98 ± 0.5* | 16.88 ± 0.3* | 37.27 ± 0.27* | 297.02 ± 6.69* |
| AE | 15 | 12.31 ± 0.02a | 96.08 ± 3.55a | 281.62 ± 11.32a | 673.3 ± 8.89a |
| | 25 | 12.48 ± 0.81a | 136.76 ± 3.35b | 391.93 ± 6.71b | 925.87 ± 24.13b |
| | 35 | 13.04 ± 0.34a | 158.62 ± 1.14c | 483.48 ± 2.51c | 1128.01 ± 23.89c |
| BV | 15 | 12.83 ± 0.02a | 55.12 ± 6.88a | 171.31 ± 7.79a | 418.74 ± 9.81a |
| | 25 | 13.83 ± 0.01b | 78.25 ± 3.57b | 317.2 ± 9.41b | 766.86 ± 10.49b |
| | 35 | 13.93 ± 0.18b | 103.2 ± 5.72c | 408.53 ± 6.72c | 965.52 ± 11.89c |
| AP | 15 | 12.45 ± 0.2a | 73.77 ± 5.07a | 216.38 ± 1.32a | 538.42 ± 17.40a |
| | 25 | 14 ± 0.34b | 114.62 ± 5.25b | 364.65 ± 12.18b | 829.63 ± 26.7b |
| | 35 | 14.38 ± 0.94b | 142.01 ± 5.66c | 463.09 ± 1.35c | 1082.99 ± 21.9c |
| GTS | 15 | 12.29 ± 0.45a | 23.21 ± 0.41a | 44.39 ± 1.06a | 407.84 ± 7.54a |
| | 25 | 13.63 ± 0.57b | 24.72 ± 0.78a | 55.06 ± 2.05b | 412.46 ± 7.84a |
| | 35 | 14.24 ± 0.04b | 27.48 ± 1.29b | 79.76 ± 1.99c | 417.08 ± 13.06a |

TP: total phenol content; FAE: ferrulic acid equivalents; AOX[1]: DPPH$^{\bullet}$ antioxidant activity; TE: trolox equivalent; AOX[2]: ABTS$^{+\bullet}$ antioxidant activity.

AE: small read bean variety AC Earlired; BV: black bean variety Black Violet; AP: pinto bean variety AC Pintoba; GTS: navy bean variety GTS 531.

All the values are Mean ± SD of four determinations adjusted to dry matter. Data followed by the same character in the same column, within the same bean flour, are not significantly different (*P*>0.05).

*Significantly different comparing to added bean flours in the same column using a two-tail t-test (*P*<0.05).

These inhibitors are thermolabile and their inhibitory activity can be reduced considerably by an appropriate thermal treatment (Alonso et al., 2000; Shimelis & Rakshit, 2007). In fact, trypsin inhibitors were reduced ranging from 50 to 66% in composite flours made into tortillas (considering only the composite flour portion of tortilla formulation, equal to 90%; i.e., excluding shortening, salt and baking powder) (Table 5).

These reductions are in agreement with the findings of Estévez et al. (1991), who found that levels of trypsin inhibitors were reduced from 57 to 62% in beans soaked for 16 h at room temperature and cooked for 60 minutes. This study reported that the cooked bean samples showed *in vitro* protein digestibility of nearly 90%, as well as good net protein ratios (3.2 in comparison to 4.2 of casein). It has been suggested that inactivation of trypsin inhibitors depends on the physical state of the material. Carvalho and Sgarbieri (1997) reported that bean flours submitted to soaking and autoclaving had significantly lower levels of inactivation than whole beans. Our results are comparable with bean flours soaked for 12 h and autoclaved at 121°C for 5 and 20 min resulting in 55 and 65% reductions, respectively. Since inactivation of trypsin inhibitors in bean tortillas has not been complete, it appears that a thermal treatment longer than that usually applied in flour tortillas manufacturing may be necessary. Nevertheless, based on the levels of substitution studied and on the degree of inactivation reached, it seems that the present bean tortillas would be safe for human nutrition.

**Table 4.5.** Effect of Added Bean Flours on Some Antinutritional Factors of Wheat Flour and Tortillas

| | | Phytic Acid (mg/10g) | | Trypsin Inhibitors (TIU/100mg) | |
|---|---|---|---|---|---|
| Flour | (%) | Raw Flour | Tortilla | Raw Flour | Tortilla |
| Wheat | 100 | 22.06 ± 0.66* | 4.95 ± 0.31* | ND | ND |
| AE | 15 | 34.68 ± 1.57a | 18.76 ± 0.83a | 222.73 ± 2.75a | 83.76 ± 3.43a |
| | 25 | 51.12 ± 2.57b | 28.07 ± 0.82b | 298.93 ± 3.8b | 128.86 ± 3.8b |
| | 35 | 69.5 ± 2.83c | 39.07 ± 0.62c | 387.35 ± 8.31c | 151.08 ± 2.59c |
| BV | 15 | 36.12 ± 1.17a | 18.15 ± 0.34a | 263.89 ± 3.4a | 77.74 ± 1.59a |
| | 25 | 55.58 ± 2.13b | 29.39 ± 0.57b | 423.24 ± 1.95b | 119.43 ± 2.4b |
| | 35 | 73.23 ± 2.25c | 41.18 ± 1.53c | 588.62 ± 6.02c | 146.64 ± 6.29c |
| AP | 15 | 37.02 ± 1.29a | 19.44 ± 0.75a | 259.59 ± 2.6a | 83.65 ± 1.5a |
| | 25 | 57.89 ± 2.83b | 31.03 ± 1.05b | 344.43 ± 5.04b | 132.51 ± 4.1b |
| | 35 | 75.89 ± 1.49c | 42 ± 1.4c | 518.7 ± 6.66c | 183.46 ± 3.94c |
| GTS | 15 | 38.71 ± 1.84a | 19.79 ± 1.36a | 364.14 ± 5.05a | 149.51 ± 2.21a |
| | 25 | 58.72 ± 2.01b | 33.78 ± 1.87b | 588.62 ± 13.52b | 207.04 ± 3.15b |
| | 35 | 77.04 ± 2.83c | 43.97 ± 1.06c | 828.9 ± 21.97c | 301.17 ± 8.13c |

TIU: trypsin inhibitory units; ND: non detectable; AE: small read bean variety AC Earlired; BV: black bean variety Black Violet; AP: pinto bean variety AC Pintoba; GTS: navy bean variety GTS 531.

All the values are Mean ± SD of four determinations adjusted to dry matter. Data followed by a different character in the same column, within the same bean flour, are significantly different ($P<0.05$).

*Significantly different comparing to added bean flours in the same column using a two-tail t-test ($P<0.05$).

All tortillas were significantly different than their respective raw flours for both phytic acid and trypsin inhibitors ($P<0.05$).

### 4.5. Conclusions

The effect of bean cultivar on some physical and nutritional properties of flour tortillas was more significant for 25% and 35% substitutions. Composite flours containing 25% bean flour demonstrated acceptable textural parameters and improved nutritional profile in comparison to the wheat control. Tortillas containing navy bean flour (GTS) had no impact on colour, however the levels of phenolics and antioxidant activity were not changed to a great extent in such formulations. Levels of antinutritional compounds were significantly reduced in all levels of substitution, indicating that although these compounds have not been entirely inactivated, bean tortillas could still be safely used for human nutrition.

Further sensory studies and biological trials must be carried out in order to evaluate the acceptability of bean tortillas by a consumer panel and to verify the impact of such foods on animal and human nutrition. Additionally, a modified manufacturing procedure and the application of texture enhancers, such as hydrocolloids, may help improve the nutritional profile and the texture of tortillas added at higher levels of bean flour. Nonetheless, our results appear to indicate that production of bean tortillas is feasible and that their consumption can play an important role in the maintenance of a healthy life style.

# 5. Shelf Stability and Sensory Properties of Flour Tortillas Fortified with Pinto Bean (*Phaseolus vulgaris* L.) Flour: Effects of Hydrocolloid Addition

## 5.1. Abstract

To tortillas containing 25% of pinto bean flour, 0.5% and 0.75% of guar gum and sodium carboxymethyl cellulose (CMC) were added and their shelf stability was studied at 4 °C and 25 °C over 7 days. Texture, determined instrumentally, rollability, and water holding capacity were the main parameters studied. Selected samples were evaluated by 55 participants to determine consumer acceptability. Firmness and cohesiveness were negatively affected by the addition of bean flour, however this effect was partially overcome by the addition of hydrocolloids. Guar gum had a positive significant influence on water holding capacity and texture over time ($P<0.001$), while CMC had no positive effects. Despite the instrumental texture data, which showed that bean tortillas had inferior attributes than the wheat control, consumers found the overall texture and acceptability of bean tortillas with and without guar gum on the range of "like very much" and "like moderately", which was significantly higher than the wheat control ($P<0.01$). Based on physical and sensorial properties it would appear that these foods are industrially feasible and highly acceptable by health-conscious consumers.

## 5.2. Introduction

In North America, the tortilla market is the fastest growing segment of the baking industry (Cornell, 1998; Kuk, 2006; Tortilla Industry Association, 2007). As part of this growth, there is the need to reconstruct the tortilla with new nutritional attributes and in new formats. Following that, the market for functional foods and the search for healthier alternatives to conventional foods, with the consumers' desire for convenience and practicability, is also increasing (Berne, 2005).

Oilseeds, isolated fibre, and alternative cereals have been reported to improve the nutritional profile of flour tortillas (Gonzalez-Agramon & Serna-Saldivar, 1988; Seetharaman et al., 1994; Serna-Saldivar et al., 2004). Considering the nutritional and economical aspects of legumes (Tharanathan & Mahadevamma, 2003; Anton et al., 2008b), the fortification of bakery goods with flours of peas, beans, and chickpeas appears to be promising (Dhingra & Jood, 2001; Doxastakis et al., 2002; Dalgetty & Baik, 2006). The common bean (*Phaseolus vulgaris* L.), in particular, has the potential to lower the high-glycemic index of flour tortillas. High in fibre, protein, and low in fat, bean consumption has been inversely associated with reduced risk of coronary diseases and some types of cancer (Azevedo et al., 2003; Winham & Hutchins, 2007). In addition, there is solid scientific evidence that coloured dry beans possess strong in vitro antioxidant activity (Beninger & Hosfield, 2003; Madhujith & Shahidi, 2005; Anton et al., 2008b), which may explain in part the protective benefits of bean consumption on development of degenerative diseases.

In our previous work (Anton et al., 2008c) we showed that tortillas fortified with 25% of flours from different bean cultivars of the 2005 crop year had acceptable textural properties and significantly improved nutritional profile in comparison to the wheat control. Among the bean cultivars studied, fortification with pinto bean flour showed a great positive impact on levels of phenolics and antioxidant activity, besides a significant increase in levels of crude protein.

Flour tortillas contain four major ingredients: flour, water, shortening/oil, and salt. In Mexico, flour tortillas contain only these ingredients, resulting in products with short shelf-life (2-4 days). However, in North America, where a far longer shelf-life is required (10-20 days), formulations contain preservatives, chemical leavening agents, emulsifiers, hydrocolloids and other ingredients to improve softness and shelf life as well as tortilla flavour and functionality (Cornell, 1998).

While consumers typically reject bread after one week on the grocery shelf, they expect tortillas to be edible over weeks and even months at a time (Friend et al., 1995). Various tortilla formulations include hydrocolloids to extend shelf life and retain freshness (Anton, 2008). Comprising a number of water-soluble polysaccharides with varied chemical structures that allow them diverse functional properties, hydrocolloids have wide applications in the food industry, including modification of starch gelatinization (Rojas et al., 1999), and extension of the overall quality of the product during time. In bakery goods, they act by improving shelf life stability and texture by retaining more moisture and retarding staling. Guar gum has been employed for improving the volume and texture of frozen dough bread (Ribota et al., 2004), while the employment of hydroxypropylmethylcellulose (HPMC) has resulted in soft bread-crumb loaves with higher specific bread volume, improved sensory characteristics and extended shelf life (Collar et al., 1998; Bárcenas & Rosell, 2005; Anton, 2008).

It is observed that as wheat flour is replaced with gluten-free materials, the texture of the bakery good is significantly affected (Anton & Arntfield, 2008; Anton et al., 2008c), which is very likely to reflect changes in their shelf-life and other sensory properties. Thus, the purpose of the current study was to examine the effect of adding two types of hydrocolloids (guar gum and sodium carboxymethyl cellulose) at two levels (0.5% and 0.75%) on some physical and sensory properties of wheat based tortillas fortified with 25% of ground pinto beans harvested in 2006. Dough rheology and characteristics of the final product such as colour, diameter, and thickness were investigated. Firmness, cohesiviness, rollability, and moisture loss were determined over time in tortillas stored at 4°C and 25°C. Sensory properties such as overall acceptability, overall texture, and flavour were determined by 55 untrained panelists.

## 5.3. Materials and Methods

### *5.3.1. General*

Pinto (variety AC Pintoba) beans were obtained from the Agriculture and Agri-Food Canada Research Station in Morden, MB, Canada. They were grown and harvested in 2006. Whole seeds were ground in a Jacobson pilot scale hammer mill (Model No 120-B, Minneapolis, MN, USA) to pass a 500 μm sieve (35 mesh US Standard Sieve Series). Pinto bean flour was added at 25% to Canadian hard red spring wheat (Laura variety, crop year 2005; 13.55% crude protein) flour. Guar gum (Food grade type FG 60-70, Multi-Kem Corporation, Ridgefield, NJ, USA) and sodium carboxymethyl cellulose (CMC) (Cellugen HP-6HS9, Multi-Kem Corporation, Ridgefield, NJ, USA) were added at 0.5% and 0.75% to composite flours and the mixtures were stored at 5 °C in opaque, closed containers for further use.

### *5.3.2. Dough Rheology*

The effect of adding hydrocolloids on dough mixing properties was determined using a Brabender Farinograph (Diusburg, Germany) according to the constant flour weight procedure (Method 54-21; AACC, 1995). The parameters determined were % water absorption, dough development time (DDT), and dough stability.

### *5.3.3. Tortillas Preparation*

100g of flour (either wheat or composites) with weight adjusted to 14% of moisture content was added to 9 g of vegetable shortening (Crisco, Markham, ON, Canada), 1.5g of baking powder (Magic Baking Powder, Kraft Canada Inc., Don Mills, ON, Canada), 1.5g of sodium chloride (Food Grade, Fisher, Ottawa, ON, Canada) and distilled water. The amount of added water was determined based on the Farinograph Water Absorption (FAB) and previous tests that determined the optimum water addition for making tortillas with the best cohesiviness possible (results not shown). The tests indicated that the final product would achieve its best by adding the FAB value minus 12 g of water. The dry ingredients plus shortening were placed onto a 200g mixer (National MFG. Co, Lincoln, NB, USA) and mixed for 2 minutes before water addition. Once water was added, the batter was further mixed for 3 min (pinto bean tortillas), or 4 minutes (wheat control). Mixing time was determined by previous tests that identified the average mixograph peak stability time for each level of substitution (results not shown). After mixing, dough was cut into pieces of 35g. These pieces were rounded, pinched on the bottom and placed in separate plastic containers, covered with a damp cloth and allowed to rest for 5 minutes at room temperature. After resting, each 35g ball was slightly flattened by hand and pressed on a hot press (Doughpro Proprocess Corporation, Paramount, CA, USA) previously heated to 93 °C (top and bottom platens) for 8s. Thereafter, tortillas were transferred to an electric hot plate preheated at 218 °C and baked for 30s on the first side, flipped and baked for 40s on the second side, and flipped and baked for another 10s on first side. Baked tortillas were cooled on a rack for 1 min and packed in open polyethylene plastic bags after their weight had been determined. The bags were sealed after 3 h and left at 4°C or 25 °C for 24, 72, or 168h (1,3, or 7 days). Colour, diameter, and thickness of tortillas were evaluated 24 h after production. Other physical properties such as firmness, cohesiveness, rollability, and moisture loss were determined over time in independent tortillas. Each batch yielded 4 tortillas.

### *5.3.4. Physical Analysis*

Tortilla firmness was determined by a puncture test with a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) equipped with a cylindrical probe (TA 108, 18 mm diameter) with a force of 40g. Tortillas were placed blistered side down on a tortilla burst rig (in accordance with the manufacturer's instructions) and firmness was measured as the resistance to puncture (peak force). Cohesiveness was calculated as the work during compression (rupture force multiplied by the distance at peak force).

Diameter was measured with a ruler and thickness with a caliper at three different places in each tortilla and the mean was calculated for each (one value was considered for each tortilla). Rollability was evaluated by wrapping a tortilla around a dowel (1.0 cm diameter) and rating the cracking and breakage of the tortilla. Rollability was rated in a scale of 1-6, where 1=no signs no cracking (best), 2=edge cracking only, 3=edge cracking and/or cracking in the center, 4=cracking and breaking on one side, 5=cracking and breaking on both sides (clean break) but still rollable, 6=unrollable.

Colour measurements (CIE $L^*$ $a^*$ $b^*$ colour space) were performed using a Minolta CM-3600d model spectrophotometer. The colour of tortillas was expressed as the average of two $L^*$, $a^*$, and $b^*$ readings, where $L^*$ stands for brightness, $+a^*$ redness, $-a^*$ greenness, $+b^*$ yellowness, and $-b^*$ blueness. A white calibration plate was used to standardize the equipment prior to colour measurements.

Moisture loss was determined by measuring the difference in weight from time zero (1 minute after cooking) to time of evaluation (day 1, 3, or 7). Values were reported by means of percentage of loss.

### *5.3.5. Sensory Evaluation*

Hedonic sensory tests were conducted by 55 untrained panelists consisting of Faculty of Human Ecology and Faculty of Agriculture & Food Sciences staff and students using the Weston Sensory and Research Centre at the University of Manitoba. For this test, the tortilla containing the hydrocolloid which had its texture most improved was chosen to be evaluated together with a pinto bean control tortilla and a wheat control tortilla.

Tortillas were evaluated on the basis of their overall acceptability, overall flavour, and texture by a hedonic 9-point scale where 9 means most liked and 1 most disliked. The bean and wheat controls were presented simultaneously with the hydrocolloid-added tortilla and were evaluated in random order among panelists. The SIMS 2000 Version 6.0 (Sensory Computer Systems, Morristown, New Jersey, U.S.A.) computer software program was utilized for this study.

Panelists were also asked to answer what types of tortilla and bread they usually consume. This information was used to verify whether there were differences in acceptability of bean tortillas for different group of consumers: those who usually eat healthy bakery goods and those used to regular refined flour products. 41 panelists declared to usually consume multi-grain, whole wheat, high-fibre and flax products; they formed the "healthy bread and tortillas consumers" group (HT). The "regular refined flour bread and tortillas consumers" group (RT) was formed by 14 panelists.

All tortillas were produced 24 h before the sensory evaluation and were stored under 4°C until cut and presented to panelists. Each panelist was given 3 types of tortilla, which were presented in one-third of a full sized tortilla (average diameter 16.4 cm).

### *5.3.6. Statistical Analysis*

All data were recorded as means ± SD and analyzed by GraphPad Instat for Windows (ver. 3). One-way analysis of variance (ANOVA), Tukey tests and two-tail t-tests were carried out to test any significant differences between tortillas and parameters studied. All tests were performed using $\alpha$=0.05.

## **5.4. Results and Discussion**

### *5.4.1. Effect of Hydrocolloid Addition on Dough Rheology*

Table 5.1 shows the effect of guar gum and CMC addition on rheological properties of wheat/bean flour doughs. In accordance to our previous work, the bean control showed significantly higher water absorption than the wheat control. This is logical since the chemical composition of beans differs from wheat. This increase may be partially explained on the basis that bean proteins are water soluble (Deshpande et al., 1983; Morales-de-Léon et al., 2007) while most of wheat proteins are water insoluble. Moreover, dough weakening could also be due to competition between bean proteins and wheat proteins for water or to the possible proteolytic activity in the dry bean flours

(Fleming & Sosulski, 1978). Similar behavior has been observed for doughs which include gluten-free flours (Gonzalez-Agramon et al., 1988; Doxastakis et al., 2002; Serna-Saldivar et al., 2004).

In comparison to the bean control, guar gum did not cause a significant impact on dough water absorption regardless of the level of addition. On the other hand, dough stability was prolonged by nearly 50% for bean dough to which 0.5% guar gum was added. CMC, however, caused greater water absorption and a substantial negative impact on dough stability. Shalini and Laxmi (2007) also reported higher water absorption for whole-wheat flour chapatti dough with added CMC in comparison to those to which guar gum was added. It has been speculated that such an effect is due to the water interactions caused through hydrogen bonding from the hydroxyl groups present in the CMC structure (Rosell et al., 2001; Shalini & Laxmi, 2007). Similar results to ours were also observed for dough stability in flour tortillas and chapattis (Indian flattened bread) formulations containing CMC (Friend et al., 1993; Shalini & Laxmi, 2007). Nevertheless, dough development time in the current study was reduced in all formulations with added hydrocolloids, which disagrees with the results reported by Friend et al. (1993) and Shalini and Laxmi (2007). Since bean flour addition causes a higher water absorption (Anton et al., 2008c) and hydrocolloids can hold as much as 100 times their weight in water (Gurkin, 2002), this may be a consequence of the interaction between bean proteins and hydrocolloids, which decrease the time necessary to reach 500 BU of dough consistency (DDT).

**Table 5.1.** Effect of Hydrocolloid Addition on Dough Rheology Properties of Pinto Bean Tortillas

| Hydrocolloid | Dosage (%) | WA (%) | DDT (min) | Stability (min) |
|---|---|---|---|---|
| Wheat Control | 0 | 65 ± 0.1a | 8.7 ± 0.2c | 13.7 ± 0.1d |
| Bean control* | 0 | 67.6 ± 0.38b | 8.7 ± 0.1c | 7.7 ± 0.19b |
| Guar gum | 0.5 | 66.7 ± 0.44b | 8.3 ± 0.17b,c | 11.3 ± 0.5c |
| | 0.75 | 66.9 ± 0.6b | 6.9 ± 0.13a | 8.1 ± 0.1b |
| CMC | 0.5 | 70.2 ± 0.35c | 7.9 ± 0.1b | 2.8 ± 0.06a |
| | 0.75 | 71.7 ± 0.6d | 8 ± 0.44b | 3 ± 0.13a |

*Composite flour containing 25% pinto bean flour and 75% Canada Western Red Spring wheat flour. Hydrocolloids were added to bean composite flours only.

WA: water absorption; DDT: dough development time.

All the values are Mean ± SD of three determinations. Data followed by the same character in the same column are not significantly different ($P$>0.05).

### *5.4.2. Effect of Hydrocolloid Addition on Some Physical Properties of Pinto Bean Tortillas*

As observed in Table 5.2, neither guar gum nor CMC caused a significant impact on diameter of pinto bean tortillas. Thickness, however, was visibly affected by hydrocolloid addition. Addition of guar gum produced bean tortillas with a degree of puffiness similar to the wheat control. In accordance with dough stability results, CMC addition resulted in thinner products, reflecting the low tolerance to mixing and poor dough structure that does not allow puffiness. It has been established that the characteristic fluffy texture of the tortilla depends upon the retention of steam and leavening gases by the gluten matrix (McDonough et al., 1996); in this sense, guar gum was able to partially mimic the gluten functionality, effectively compensating for the weak structure caused by substituting 25% of wheat flour with bean flour. Many types of hydrocolloids have been successfully applied in the production of gluten-free breads (Anton & Arntfield, 2008).

Hydrocolloid addition caused a significant impact on colour. Both guar gum and CMC produced tortillas that were brighter and in general more similar to the wheat control than the bean control, which is a positive effect on their visual appearance.

Tables 5.3 and 5.4 show the effect of hydrocolloid addition on the shelf stability of pinto bean tortillas evaluated over time, as well as the significance of such additions on different days and temperatures for the diverse parameters studied. In all types of tortillas, firmness and cohesiveness decreased significantly from day 1 to day 7. This is clearly visible for tortillas stored at room temperature (25°C), which also had their rollability negatively affected on day 7, except for those to which 0.75% guar gum was added. Storage in low temperature (4°C) led to tortillas that were more rollable and consistently cohesive over time.

**Table 5.2.** Effect of Hydrocolloid Addition on Some Physical Properties of Pinto Bean Tortillas Evaluated on Day 1*

| Hydrocolloid | Dosage (%) | Diameter (mm) | Thickness (mm) | Colour | | |
|---|---|---|---|---|---|---|
| | | | | *L** | *a** | *b** |
| Wheat Control | 0 | 15.7 ± 0.2a | 1.73 ± 0.1c | 83.23 ± 0.3d | -0.53 ± 0.19a | 23.65 ± 0.14c |
| Bean control** | 0 | 16.55 ± 0.05b,c | 1.45 ± 0.06a,b | 70.21 ± 0.21a | 2.2 ± 0.1b | 16.06 ± 0.21a |
| Guar gum | 0.5 | 16.5 ± 0.06a,b | 1.63 ± 0.05b,c | 75.03 ± 0.3c | 1.94 ± 0.21b | 15.78 ± 0.42a |
| | 0.75 | 16.28 ± 0.19b | 1.6 ± 0.08b,c | 74.44 ± 0.86c | 2.22 ± 0.27b | 16.09 ± 0.81a |
| CMC | 0.5 | 16.83 ± 0.1c | 1.35 ± 0.17a | 72.56 ± 0.28b | 3.98 ± 0.17d | 16.29 ± 0.61a |
| | 0.75 | 16.7 ± 0.26c | 1.45 ± 0.1a,b | 72.59 ± 1.01b | 2.78 ± 0.38c | 17.71 ± 0.86b |

* 24 h after manufacturing

*L**: brightness; *a**: greenness(-)/redness (+); *b**: blueness (-)/yellowness (+).

**Tortilla made out of composite flour containing 25% pinto bean flour and 75% Canada Western Red Spring wheat flour. Hydrocolloids were added to bean composite flours only.

All the values are Mean ± SD of three determinations. Data followed by the same character in the same column are not significantly different ($P$>0.05).

**Table 5.3.** Effect of Hydrocolloid Addition on Some Physical Properties of Pinto Bean Tortillas Evaluated Over Time

| Hydrocolloid | (%) | Day | Temperature (°C) | Firmness (g) | Cohesiveness (g/s) | Rollability |
|---|---|---|---|---|---|---|
| Wheat | — | 1 | 4 | 836 ± 47b | 6361 ± 84c | 1a |
| | | | 25 | 896 ± 5c | 5377 ± 82b | 1a |
| | | 3 | 4 | 971 ± 31d | 6897 ± 68d | 1a |
| | | | 25 | 994 ± 14d | 6475 ± 66c | 1a |
| | | 7 | 4 | 1021 ± 4d | 6911 ± 50d | 1a |
| | | | 25 | 601 ± 21a | 2394 ± 49a | 1.5 ± 0.57b |
| Bean | — | 1 | 4 | 746 ± 18c | 3617 ± 50c | 1a |
| | | | 25 | 717± 32b,c | 3249 ± 89b | 1a |
| | | 3 | 4 | 714 ± 72b,c | 3523 ± 130c | 1a |
| | | | 25 | 597 ± 28a | 2043 ± 88a | 1a |
| | | 7 | 4 | 661 ± 17a,b,c | 3933 ± 47d | 1a |
| | | | 25 | 639 ± 60a,b | 2042 ± 125a | 2.25 ± 0.5b |
| Guar gum | 0.5 | 1 | 4 | 792 ± 42a,b | 4206 ± 196e | 1a |
| | | | 25 | 766 ± 12a,b | 3722 ± 63d | 1a |
| | | 3 | 4 | 758 ± 20a,b | 3631 ± 135c | 1a |
| | | | 25 | 736 ± 24a | 2866 ± 105a | 1a |
| | | 7 | 4 | 814 ± 38b | 4008 ± 79e | 1a |
| | | | 25 | 774 ± 42a,b | 3168 ± 137b | 1.25 ± 0.5b |
| | 0.75 | 1 | 4 | 730 ± 36a | 4502 ± 93d | 1a |
| | | | 25 | 718 ± 43a | 3673 ± 154c | 1a |
| | | 3 | 4 | 751 ± 20a | 3854 ± 57c | 1a |
| | | | 25 | 722 ± 16a | 2963 ± 106a | 1a |
| | | 7 | 4 | 711 ± 38a | 3284 ± 134b | 1a |
| | | | 25 | 732 ± 54a | 3123 ± 77a,b | 1a |
| CMC | 0.5 | 1 | 4 | 676 ± 7c | 3734 ± 7d | 1a |
| | | | 25 | 688 ± 59c | 3542 ± 97c,d | 1a |
| | | 3 | 4 | 648 ± 55b,c | 3370 ± 184c | 1a |
| | | | 25 | 576 ± 30a,b | 2222 ± 104b | 1a |
| | | 7 | 4 | 533 ± 7a | 1971 ± 33a | 1a |
| | | | 25 | 633 ± 14b,c | 2131 ± 57a,b | 1.5 ± 0.57b |
| | 0.75 | 1 | 4 | 566 ± 30b,c | 2907 ± 147c | 1a |
| | | | 25 | 611 ± 40c | 2919 ± 80c | 1a |
| | | 3 | 4 | 607 ± 11c | 2952 ± 95c | 1a |
| | | | 25 | 523 ± 17a | 2170 ± 90b | 1a |
| | | 7 | 4 | 541 ± 31a,b | 2327 ± 50b | 1a |
| | | | 25 | 510 ± 22a | 1795 ± 54a | 1.75 ± 0.5b |

*Tortilla made out of composite flour containing 25% pinto bean flour and 75% Canada Western Red Spring wheat flour. Hydrocolloids were added to bean composite flours only.

All the values are Mean ± SD of four determinations. Data followed by the same character in the same column, within the same flour and level of hydrocolloid, are not significantly different ($P$>0.05).

**Table 5.4.** Effect of Type and Concentration of Hydrocolloid on Some Physical Properties of Pinto Bean Tortillas Evaluated Over Time Under Different Temperatures[1]

| | Day | °C | Firmnes | Cohesivenes | Rollability | Moisture Loss |
|---|---|---|---|---|---|---|
| Guar gum | | | | | | |
| 0.5% | 1 | 4 | NS | ** | NS | * |
| | | 25 | NS | *** | NS | ** |
| | 3 | 4 | NS | NS | NS | ** |
| | | 25 | *** | *** | NS | * |
| | 7 | 4 | *** | NS | NS | *** |
| | | 25 | * | *** | * | ** |
| 0.75% | 1 | 4 | NS | *** | NS | ** |
| | | 25 | NS | *** | NS | *** |
| | 3 | 4 | NS | ** | NS | *** |
| | | 25 | *** | *** | NS | ** |
| | 7 | 4 | * | *** | NS | *** |
| | | 25 | NS | *** | ** | ** |
| CMC | | | | | | |
| 0.5% | 1 | 4 | *** | ** | NS | NS |
| | | 25 | NS | * | NS | * |
| | 3 | 4 | NS | NS | NS | NS |
| | | 25 | NS | * | NS | NS |
| | 7 | 4 | *** | *** | NS | NS |
| | | 25 | NS | NS | NS | NS |
| 0.75% | 1 | 4 | *** | *** | NS | NS |
| | | 25 | ** | ** | NS | NS |
| | 3 | 4 | * | *** | NS | NS |
| | | 25 | ** | NS | NS | NS |
| | 7 | 4 | *** | *** | NS | NS |
| | | 25 | ** | * | NS | * |

[1]In comparison to the bean control tortilla (made out of composite flour containing 25% pinto bean flour and 75% Canada Western Red Spring wheat flour) using a two-tail t-test. NS: no significant effect ($P$>0.05); * $P$<0.05; ** $P$<0.01; ***$P$<0.001.

The most important physical parameters determined instrumentally in the shelf stability and quality of tortillas are cohesiveness, rollability and moisture loss (Friend et al., 1992; Friend et al., 1993; Friend et al., 1995; Anton et al., 2008c). Guar gum addition resulted in significant improvement in cohesiveness, rollability on day 7, and moisture loss (Table 4). A more visible improvement was observed for tortillas stored at room temperature. The beneficial effects of hydrocolloids in tortilla processing have been discussed by Gurkin (2002). The major functions of hydrocolloids and their interactions were reviewed, and the author concluded that water-binding is the main feature of gums in tortillas. The ability of the large molecules to hold moisture was reported to help prevent staling. Moreover, the interactions between different structures were emphasized. In this regard, it cited the beneficial combination of guar gum with CMC as water-binding agents, confirming their potential as textural improvers.

Friend and colleagues (1993) studied the addition of natural gums (Arabic, guar, and xantham), modified cellulose (CMC, HPMC and methylcellulose) and commercial blends (mixtures of natural and modified cellulose gums) of hydrocolloids on processing and qualities of flour tortillas. It was found that tortillas with added hydrocolloids were consistently round, puffed, slightly browned, and of good quality. Texture was verified by determining rollability over time, and tortillas containing CMC and cellulose-based commercial blends were reported to retain their rollability for longer. During freezing and thawing, rollability of all tortillas decreased, however those containing CMC were significantly more rollable than the control after five freeze-thaw cycles.

In our study CMC exhibited a significant negative impact on the shelf stability parameters evaluated. In general, CMC addition could not prevent moisture loss in any of our experimental conditions. Conversely, firmness and cohesiveness of tortillas added of CMC at both levels were significantly reduced, demonstrating that this hydrocolloid does not appear to be a good texture improver for the bean tortillas studied. Although dough water absorption for bean-wheat composite flours containing CMC was significantly higher than the others, the physical analysis of the final product indicates that this hydrocolloid was not able to hold all the water taken up by the flour. Figure 5.1 illustrates the moisture loss of tortillas determined over time. Guar gum could visibly retain more water than CMC and the bean control in both storage conditions. CMC, nonetheless, allowed similar moisture losses as the bean control.

Recently, the influence of hydrocolloids on rheological characteristics of whole-wheat dough and quality of Chapatti has been reported (Shalini & Laxmi, 2007). In this study, guar gum, CMC, HPMC, and k-carrageenan were investigated in relation to the textural characteristics of fresh and stored chapattis. Hydrocolloids were incorporated at various levels ranging between 0.25% and 1.0% w/w of whole wheat flour. Amongst the hydrocolloids studied, they observed that guar gum gave the highest extensibility for fresh and stored chapatti. The force required to tear the fresh chapatti decreased with hydrocolloid addition, however guar gum addition at 0.75% w/w of whole wheat flour gave the softest chapatti. Extensibility of stored chapatti significantly decreased with storage both at room as well as refrigeration temperature, although refrigerated chapatti containing guar gum showed less loss in extensibility up to a period of 2 days.

From our results, guar gum was also the most effective in producing tortillas with extended shelf stability and overall quality. Considering dough rheology and the physical properties investigated, guar gum added at 0.5% would be the most cost-effective for industrial applications. Thus, this formulation was chosen, together with the wheat and pinto bean controls, to be tested for their sensory properties.

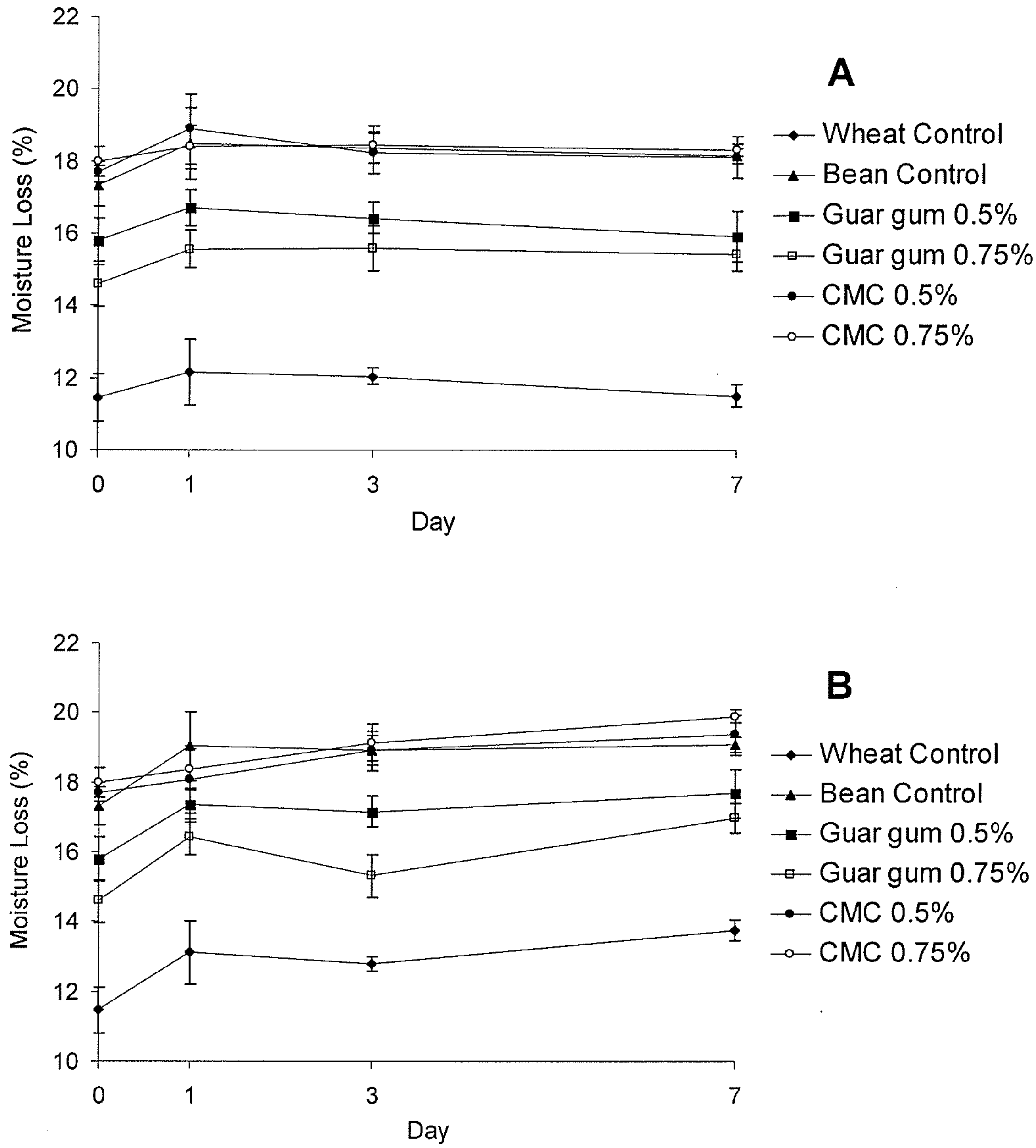


**Figure 5.1.** Moisture Loss of Bean Tortillas Stored at 4 °C (**A**) and 25 °C (**B**) Evaluated Over Time (time zero corresponds to moisture loss measured 1 minute after cooking)

### *5.4.3. Effect of Hydrocolloid Addition on Some sensory Properties of Pinto Bean Tortillas*

Table 5.5 shows the sensory scores of tortillas made out of wheat flour alone (wheat control), wheat fortified with 25% of pinto bean flour (bean control), and wheat fortified with 25% of pinto bean flour plus addition of 0.5% of guar gum (bean GG). Overall acceptability of bean tortillas was, for the total population, significantly higher than the wheat control. No significant difference was observed for bean tortillas containing 0.5% guar gum in comparison to the bean control. Breaking down the results from the 55 panelists to those 41 who declared a preference for healthy bread and tortillas (HT group), overall acceptability scores remained the same as the total population. However, by analyzing the data obtained from the 14 panelists who declared to usually eat regular white flour bread and tortillas (RT group), no significant difference was found among the types of tortillas evaluated. Conversely, the overall acceptability of bean tortillas containing 0.5% guar gum was significantly higher for the HT group in comparison to the RT group. This makes sense since consumers who are used to consuming multi-grain, whole wheat, high-fibre, and flax bakery goods are more likely to accept different texture, appearance, and flavour in a new nutritious product.

Overall flavour and texture of both bean tortillas had in general higher acceptability than the wheat control. No significant difference in these parameters was observed among tortillas evaluated by the RT group. Nonetheless, texture of bean tortillas with added guar gum was significantly more acceptable ($P$<0.001) by panelists from the

HT group. In this regard, instrumental data poorly reflected the data obtained from the sensory evaluation.

**Table 5.5.** Sensory Scores of Selected Tortillas

| | Population (n) | Wheat control | Bean control[1] | Bean GG[2] |
|---|---|---|---|---|
| Overall acceptability | Total (55) | 5.8 ± 1.71a | 7.18 ± 1.29b | 6.96 ± 1.36b |
| | HT[3] (41) | 5.77 ± 1.89a | 7.34 ± 1.16b | 7.14 ± 1.23b* |
| | RT[4] (14) | 5.73 ± 2.28a | 6.55 ± 1.63a | 6.27 ± 1.68a |
| Overall Flavour | Total | 5.73 ± 1.84a | 6.95 ± 1.41b | 7 ± 1.47b |
| | HT | 5.77 ± 1.89a | 6.98 ± 1.37b | 7.2 ± 1.37b* |
| | RT | 5.55 ± 1.69a | 6.82 ± 1.6a | 6.18 ± 1.6a |
| Texture | Total | 6.11 ± 1.96a | 7.36 ± 1.37b | 7.24 ± 1.29b |
| | HT | 6.23 ± 1.92a | 7.45 ± 1.3b | 7.52 ± 1.07b*** |
| | RT | 5.64 ± 2.16a | 7 ± 1.61a | 6.09 ± 1.51a |

[1]Tortilla made out of composite flour containing 25% pinto bean flour and 75% Canada Western Red Spring wheat flour.

[2]Tortilla made out of composite flour containing 25% pinto bean flour and 75% Canada Western Red Spring wheat flour added of guar gum at 0.5%.

[3]HT: Participants who declared to usually consume healthy bread and tortillas (multi-grain, whole wheat, high-fibre and flax).

[4]RT: Participants who declared to usually consume regular white flour bread and tortillas.

Data followed by the same character in the same row are not significantly different ($P$>0.05).

*Significantly different comparing to scores of the RT population, considering the same type of tortilla for the same sensory attribute, using a two-tail t-test (*$P$<0.05, ***$P$<0.001).

Although the wheat control had the best cohesiveness and rollability amongst the tortillas studied, it was the combination of all their organoleptic properties that accounted for their acceptability by consumers. Addition of guar gum improved cohesiveness, rollability and moisture loss of bean tortillas over time, however such improvement was not perceived when tortillas were evaluated 24 hours after production.

### 5.5. Conclusions

Hydrocolloid addition caused a significant impact on dough rheology, shelf stability, and some physical properties of pinto bean tortillas. CMC addition resulted in poorer dough tolerance to mixing and decreased shelf stability, while guar gum caused a positive impact on these parameters. On the other hand, the beneficial effects of combining guar gum and CMC on improving kneading behavior of tortilla dough and product strength and pliability even after freezing and heating have been reported (Gurkin, 2002; Qarooni, 1996).

Guar gum addition at 0.5% did not affect acceptability of bean tortillas, which were, in general, significantly more acceptable than the wheat control. Since one of the major problems in tortilla quality is the deterioration of texture with time due to staling (Waniska, 1999), guar gum proved to contribute towards a more shelf stable product, indicating to be a potential additive in bean tortilla formulations. Our results show that there is a market opportunity for introducing new types of tortillas in the North American market, and that fortification of flour tortillas with 25% of pinto bean flour is feasible and acceptable. Although it appears that bean tortillas would appeal to health-conscious consumers, research must be continued in this area for the achievement of adequate formulations for the preparation of high quality products on a large scale.

## 6. Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (*Phaseolus vulgaris* L.) flour: Effects of bean addition and extrusion cooking

### 6.1. Abstract

Navy and red bean flours (BF) were added to corn starch at levels of 15, 30, and 45% and submitted to extrusion cooking to produce fortified puffed snacks. Process variables (screw speed, moisture, and temperature of the final zones) of a twin screw extruder were kept constant (150 rmp, 22% and 160°C). Corn starch-bean extrudates were denser, less expanded, and harder. However starch fortified with 30% BF produced extrudates with percentage of deformation – an instrumental measurement of crispness-comparable to corn starch alone. At this level, crude protein was increased 12-fold, while total phenols, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) and Oxygen Radical Absorbance Capacity (ORAC) *in vitro* antioxidant activities (AA) were also increased. Red bean fortification yielded extrudates with higher levels of phenols and both DPPH$^\bullet$ and ORAC AA compared to navy beans. In navy and red bean extrudates, total phenols, DPPH$^\bullet$, and ORAC AA were reduced in 10, 17, and 10%, and in 70, 62, and 17% after extrusion, respectively. Phytic acid and trypsin inhibitors levels were reduced in nearly 50 and 100% in all bean extrudates compared to raw mixtures, indicating that these materials were safe for human consumption.

## 6.2. Introduction

Extrusion cooking is an important processing technique in the food industry as it is considered to be an efficient manufacturing process. Food extruders provide thermo-mechanical and mechanical energy (shear) necessary to cause physico-chemical changes of raw materials with an intense mixing for dispersion and homogenization of ingredients (Linko et al., 1981; Wiedman & Strobel, 1987; Anton & Luciano, 2007).

Extruded foods are composed mainly of cereals, starches, and/or vegetable proteins. The major role of these ingredients is to give structure, texture, mouth feel, bulk, and many other characteristics desired for specific finished products (Launey & Lisch, 1983; Tahnoven et al., 1998). Consumer acceptance of extruded foods is mainly due to the convenience, value, attractive appearance and texture found to be particular for these foods, especially when it concerns snack products (Harper, 1981; Anton & Luciano, 2007).

While corn starch provides all the features for production of highly acceptable extruded snack foods, its nutritional value is far from satisfying the needs of health-conscious consumers (Rampersad et al., 2003). Several attempts to improve the nutritional profile of extruded starch have been reported (Liu et al., 2000; Onwulata et al., 2001; Rampersad et al., 2003). Among other materials, incorporation of legume flours has been shown to cause a positive impact on levels of proteins and dietary fibre of corn starch-based extruded snacks (Berrios, 2006). On the other hand, addition of high-fibre, high-protein alternate ingredients to starch has been demonstrated to significantly affect the texture, expansion and overall acceptability of extruded snacks (Liu et al., 2000;

Veronica et al., 2006). For the production of nutritious acceptable snacks, rates of starch fortification seem to vary according to the nature of each material. Legumes, for example, have been reported to cause good expansion and are regarded as highly feasible for the development of high-nutritional, low-calorie snacks (Berrios, 2006).

Taking into account the nutritional and economical aspects of common beans (*Phaseolus vulgaris* L.) (Tharanathan & Mahadevamma, 2003; Anton et al., 2008b), fortifying corn starch with flours and fractions of varied bean cultivars for the production of extruded snacks appears to be promising. High in fibre, protein, and low in fat, bean consumption has been inversely associated with reduced risk of coronary diseases and some types of cancer (Azevedo et al., 2003; Winham & Hutchins, 2007). In addition, there is solid scientific evidence that coloured dry beans possess strong *in vitro* antioxidant activity (Beninger & Hosfield, 2003; Madhujith & Shahidi, 2005; Anton et al., 2008b), which may explain, in part, the protective benefits of bean consumption on development of degenerative diseases.

Antioxidants in beans are related to the presence of phenolic compounds that influence their seed coat colour (Beninger et al., 2003; Madhujith et al., 2005). In this regard, coloured dry beans such as red, pinto and black, are expected to possess stronger antioxidant activity than navy beans. Although little is known about the effect of extrusion cooking on phenolic composition and antioxidant activity of dry beans (Korus et al., 2007a, b), thermal processing of beans has been reported to cause important changes on these parameters (Rocha-Guzmán et al., 2007; Anton et al., 2008b).

Additionally, extrusion cooking has been used to partially or totally inactivate several antinutritional compounds that limit the widespread use of beans as a primary staple food (Alonso et al., 2000; Shimelis & Rakshit, 2007). These compounds, such as phytic acid and trypsin inhibitors, might produce adverse effects for human and animal nutrition (Martin-Cabrejas et al., 2004). Extrusion has also been reported to be the most effective method for improving protein and starch digestibility of kidney beans extrudates (Alonso et al., 2000, Berrios, 2006). Consequently, fortification of corn starch with bean flour is believed to add value to dry beans as well to as result in a product with high nutritional appeal.

This work aimed to determine the technical feasibility of adding varied levels of navy and red bean flour (15, 30, and 45%) to corn starch for production of puffed snack foods through extrusion, as well as to examine the effect of extrusion cooking on levels of nutritional and antinutritional compounds of the various formulations. Parameters such as bulk density, expansion ratio, breaking strength and deformation were used to evaluate the physical properties of extrudates. They were aimed to reflect the technical feasibility of incorporating bean flours into corn-based extruded materials. Additionally, levels of protein, antioxidants, total phenolics, trypsin inhibitors, and phytic acid were measured to determine the nutritional impact of bean fortification and to assess the consequences of the thermal treatment on these parameters.

## 6.3. Materials and Methods

### *6.3.1. Acquisition of Samples and Preparation of Flours*

Navy (variety GTS 531) and small red (variety AC Earlired) beans were obtained from the Agriculture and Agrifood Canada Research Station in Morden, MB, Canada. The cultivars were grown and harvested in 2006 and exposed to the same environmental conditions in order to avoid external variation. The weight of 100 seeds was determined gravimetrically and expressed as mean ± SD of 3 determinations. Crude protein (AOAC, 1991) content of bean samples were: 24.06% for navy, and 21.27% for small red beans.

Whole seeds were ground in a Jacobson pilot scale hammer mill (Model No 120-B, Minneapolis, USA) to pass a 500 μm sieve (35 mesh US Standard Sieve Series). Ground samples were added at different levels (15, 30, and 55%) to regular corn starch (moisture 9.8%, 25% amylose 75% amylopectin - Casco, Etobicoke, ON, Canada) and the composite flours were stored at 5 °C in opaque, closed containers for further use. The raw composite flours, as well as the extruded products, were analyzed for their moisture content by AOAC method 925.10 (AOAC, 1990).

### *6.3.2. Extrusion*

A laboratory scale twin screw extruder (MPF 19:25, APV Baker Inc., Grand Rapids, MI, USA) under high shear and high temperature in the final zones was used. The barrel diameter was 19.0 mm and the screw configuration with a length to diameter (*L/D)* ratio of 25.0 is given below:

8 *D* Feed screws

6 x 30° Forward kneading paddles

6 *D* Feed Screws

1 x Kneading paddle

1 *D* Single lead screw

2 x 60° Forward kneading paddles

2 x 60° Reverse kneading paddles

1 *D* Single lead screw

3 x 60° Forward kneading paddles

1 *D* Single lead screw

2 x 60° Forward kneading paddles

4 x 60° Reverse kneading paddles

3 *D* Single lead screws

Screw diameter = 19.00 mm (1 *D*)

One kneading paddle = ¼ *D*

Composite flours were added to the feed hopper and deionized water was injected as the mixture reached the screw zone, allowing a fixed feed moisture of 22%. Based on preliminary experiments, the following conditions were kept constant: 150 rpm screw rotation, 1.8 Kg/h feed rate, 4.5mm die diameter. The barrel consisted of five independent zones, electrically heated and cooled by water. Barrel temperature zones profile was set to 30/80/120/160/160°C. Extruded products were cooled for 30 min in room temperature and then placed in sealed plastic bags for 24h in room temperature. Extrudates were analyzed for their physical properties 24 h after production. The most appropriate feed moisture (22%) and temperature (160°C) for production of the most expanded extrudates observed for our flours are in accordance with the findings of Balandrán-Quintana et al., (1998), whose work reported the best extrusion conditions for production of extruded whole pinto bean meal.

### *6.3.3. Physical Analysis*

Expansion ratio was determined as the diameter of extrudates divided by the diameter of the die exit (4.5mm) (Gujska & Khan, 1991). Diameters at 3 different locations along the 40mm strand of an extrudate were measured first and the expansion ratio was calculated by dividing the average diameter of the strand in mm by 4.5. The specific length of extrudates was evaluated as their straight length divided by the equivalent weight of each individual strand (Alvarez-Martinez et al., 1988). Density (ρ) was determined following the method of Wang et al. (1993) by measuring the diameter (*d*), length (*l*) and weight (*Pm*) of each extrudate. It was calculated as

$$\rho = \frac{Pm}{\pi(d/2)^2 l}$$

Mechanical properties of extrudates were determined through a three point bending test using a Zwick Z005 materials testing machine (Zwick USA, Kennesaw, GA, USA) equipped with a 1 KN load cell and a Warner-Bratzler shear cell (1 mm thick blade). Tests were controlled and data were compiled using the software TextXpert II

(Zwick GmbH, Ulm, Germany). The extrudates were analyzed at a cross head speed of 0.2 mm/s. Breaking strength index (BSI) was calculated using: BSI= Peak breaking force (N)/ extrudate cross-sectional area ($mm^2$). dL (Fmax) was defined as deformation at maximum force, meaning how much the shear cell penetrated the sample until breaking. This information was used to calculate the % Deformation, defined as dL (Fmax) x 100 / extrudate diameter. For all physical analysis so far described, at least ten strands of each type of extrudate were assayed for each test. Following the described measurements, extrudates were ground in a coffee grinder (Smart Grind, Black & Decker, USA) so that the meal passed through a 500 μm sieve (35 mesh US Standard Sieve Series). The ground samples were stored at 5 °C for no more than 3 weeks in opaque, closed containers.

Colour measurements (CIE $L^*$ $a^*$ $b^*$ colour space) were performed on ground samples using a Minolta CM-3600d model spectrophotometer. The colour of extrudates was expressed as the average of three $L^*$, $a^*$, and $b^*$ readings, where $L^*$ stands for brightness, $+a^*$ redness, $-a^*$ greenness, $+b^*$ yellowness, and $-b^*$ blueness. A white calibration plate was used to standardize the equipment prior to colour measurements. Chemical analyses were performed on ground samples only after they were warmed to room temperature.

### *6.3.4. Chemical Analysis*

Nitrogen content was determined by using the Kjeldahl method and was multiplied by a factor of 5.7 to estimate protein content (AOAC, 1990).

Total phenol content and antioxidant activity were determined in both raw and cooked mixtures. For such determinations, 100 mg of finely ground sample was extracted in 2.5 mL of acetone/water (80:20, v/v) (Fisher, Ottawa, ON, Canada) for 2 h in a rotary shaker. After this period, the samples were centrifuged at 3,000 x g in a table centrifuge (GLC-1, Sorval, Newton, CT, USA) for 10 min. Thereafter the supernatant was transferred to a 3 ml syringe (Fisher) and filtered through a 0.45 μm sterile PVDF filter unit (Fisher). The filtrate was collected for further analysis.

The total phenolic content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965) as modified by Gao et al. (2002). An aliquot (0.2 mL) of extract was added to 1.5 mL of freshly diluted 10-fold Folin- Ciocalteau reagent (BDH, Toronto, ON, Canada). The mixture was allowed to sit for 5 min and then 1.5 mL of sodium carbonate solution (60 g/L) (Sigma, St Louis, MO, USA) was added. Afterwards, the mixture was incubated for 90 min and the absorbance read at 725 nm. Acetone/water (80:20, v/v) was used as a blank and ferulic acid (Sigma) was used as the standard. The results were expressed in mg of ferulic acid equivalents per 100 g of sample. Linearity range of the calibration curve was 20 to 200 μg ($r$=0.99).

For measuring the antioxidant activity two methods were employed. Antioxidant activity was initially measured using a modified version of Chen and Ho (1995). For this assay, 200 μL of extract was reacted with 3.8mL of 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) (Fisher) solution ($6.34 \times 10^{-5}$ M in methanol). The decreasing absorbance was monitored at 517 nm (Ultraspec 200, Pharmacia Biotech Piscataway, NJ) in the dark at 30 minutes against a methanol blank. The control consisted of 200 μL of acetone/water

(80:20, v/v) in 3.8 mL of DPPH$^{\bullet}$ solution. The results were obtained as a percent of discolouration according with the formula:

$$\left[1-\left(\frac{Absorbance\ Sample}{Absorbance\ Control}\right)\right]X\,100$$

Simultaneously to the samples, 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) (Sigma) was used as a standard and the results were expressed as µmol of Trolox equivalents per 100 g of sample. The linear range of the calibration curve was 2.5 to 20 µmol ($r$=0.99).

The second method for determining antioxidant activity was the oxygen radical absorbance capacity (ORAC) assay according to the procedures described by Huang et al. (2002) as modified by Li et al. (2007). An FLx800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT) was used with fluorescence filters for an excitation wavelength of 485/20 nm and an emission wavelength of 528/20 nm. The plate reader was controlled by KC4 3.0 software (version 29). Samples, rutin control, and the Trolox standard were diluted manually. 300 µL of buffer solution (blank), diluted sample, rutin control, or Trolox standard was transferred to a 96 well flat-bottom polystyrene microplate (Corning Incorporated, Corning, NY) by hand according to their designated positions. A full automation of plate-to-plate liquid transfer was programmed by using a Precision 2000 microplate pipetting system (Bio-Tek Instruments, Inc., Winooski, VT). A peroxyl radical was generated by (2,2'-azobis(2-aminopropane) dihydrochloride) (AAPH) during measurement, and fluorescein was used as the substrate. Final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the fluorescence decay curve. The area under curve (AUC) was calculated as

$$AUC = 0.5 + f_1/f_0 + \ldots + f_i/f_0 + \ldots + f_{49}/f_0 + 0.5(f_{50}/f_0)$$

where $f_0$ = initial fluorescence reading at 0 min and $f_i$ = fluorescence reading at time $i$ min. The net AUC was obtained by subtracting the AUC of the blank from that of the sample. ORAC values were expressed as Trolox equivalents according to the standard curve. Final results were expressed as mg TE per 100 g of sample.

Phytic acid levels were determined by the method of Latta and Eskin (1980). This analysis was done with a chromatographic column (0.7 cm × 15 cm) containing 0.5 g of an anion-exchange resin (100–200 mesh, chloride form; AG1-X8, Bio-Rad Co.). The Wade reagent (1 mL, 0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid in distilled water) was added into the extract (3 mL), and the mixture vortexed for 30s. The absorbance of the supernatant was measured at 500 nm with a UV-VIS spectrophotometer.

Trypsin inhibitor activity was measured following the procedure by Kakade et al. (1974), using α-*N*-benzoyl-DL-arginine-*p*-nitroanilidehydrochloride (BAPNA) (Sigma) as the substrate for trypsin. 500 mg of finely ground sample was extracted with 25 mL of 0.01N sodium hydroxide for 3 hours at room temperature in a rotatory shaker. Extracts were centrifuged at 17,500 x g (RC5C, Sorval, Newton, CT, USA) at 4°C for 20 min, and the supernatants filtered through #1 Whatman filter paper. Thereafter extracts were diluted to 30% in distilled water so that 1.0 mL could inhibit 50% of trypsin activity in the conditions presented herein. Five portions of extracts (0, 0.6, 1.0, 1.4, and 1.8 mL) were pipetted into test tubes and the final volume was adjusted to 2 mL with distilled water. Trypsin solution (2 mL, 20 mg/L in 0.001M HCl) was added and the tubes were placed in the water bath at 37°C, followed by addition of 5 mL of N-a-benzoyl-DL-arginine-p-nitroanilide (BAPNA) solution (0.4 mg/mL in Tris-buffer 0.05 M, pH 8.2) previously warmed to 37°C. After exactly 10 min the reaction was stopped by adding 1 mL of 30% acetic acid to each test tube. The absorbance was read at 410 nm and the reagent blank prepared by adding 1 mL of 30% acetic acid to a test tube containing trypsin and water (2 mL of each) before the BAPNA solution was added. One trypsin unit was arbitrarily defined as an increase of 0.01 absorbance unit at 410 nm per 10 mL of the reaction mixture under the conditions used herein.

### *6.3.5. Statistical analysis*

All data were recorded as means ± SD and analyzed by GraphPad Instat for Windows (ver. 3). One-way analysis of variance (ANOVA), Tukey tests and two-tail t-tests were carried out to test any significant differences between treatments and cultivars. Pearson's correlation coefficient (*r*) was also applied to establish specific correlations. All tests were performed using $\alpha=0.05$.

## 6.4. Results and Discussion

### *6.4.1. Effect of Bean Flour Addition on Some Physical Properties of Corn Starch-based Extrudates*

Table 6.1 summarizes the impact of added bean flour on some physical properties of corn starch extrudates. As expected, increasing levels of bean flour resulted in a significant decrease in expansion. By decreasing the amount of corn starch in the mixtures and increasing the concentration of protein and fibre through addition of bean flour, less expanded products were formed due to interactions between these components and the starch. This lower expansion can also be explained on the basis that fibre can rupture cell walls and prevent air bubbles from expanding to their maximum potential (Pérez-Navarrete et al., 2006). Such an argument may also support the fact that navy bean flour fortification produces slightly less expanded products than small red beans. This was probably due to the higher amount of fibre from the seed coats in the flour of navy beans, which is based on the weight of 100 seeds (Navy: 15.17 ± 0.41g; Small red: 27.21 ± 1.83g); when the mass of the seeds is less, the seed coat comprises a larger area relative to one whole seed. Similar behavior has been reported for maize and Lima bean flour extrudates (Pérez-Navarrete et al., 2006).

Density of extrudates increased significantly with bean flour addition, except for fortification with 15% of small red bean flour, which did not significantly affect this parameter. There is solid evidence in the literature that as high-fibre, high-protein materials are added to starch-based extruded products, density is expected to increase

(Onwulata et al., 2001; Veronica et al., 2006). In our study, this parameter was inversely correlated with expansion ratio ($r$= -0.89, $P$<0.01) based on the same rationale. Gujska et al. (1991) suggested that the degree of expansion affects the density, fragility and overall texture of extruded products.

The specific length of extrudates correlates their length with their weight as an expression of axial expansion. Negative correlations between radial and axial expansion ratios have been reported (Launay et al., 1983; Alvarez-Martinez et al., 1988), which agrees with our findings ($r$= -0.88, $P$<0.01).

Table 6.2 shows the effect of bean cultivar on the properties herein studied. Cultivar effects were present at all levels of bean flour substitution for colour, phenolic content and antioxidant activity. Fortification with navy bean flour caused a slight impact on colour, while small red bean flour addition resulted in clearly seen colour changes. Nonetheless, as there is a market opportunity for introducing new food products with high-nutritional appeal, the health-conscious consumer is likely to accepted different organoleptic features such a appearance, flavour, and texture (Anton et al., 2008a).

Physical properties of extrudades were negatively affected by added bean flours (Table 6.1). While breaking strength index increased with higher levels of bean flour substitution, instrumental parameters aimed to reflect the crispness of extrudates were decreased. The distance traveled by the shear cell until total breakage of extrudate, together with the corresponding percentage of deformation of each extrudate, were taken as a measure of crispness. A crispier product is reflected by longer distances traveled by the shear cell at maximum force (dL), higher percent deformation and a larger number of major peak forces during analysis of the puffed extrudates. Higher deformation as a result of many fracture events is regarded as a consequence of crispy extruded products (Roudaut et al., 2002; Veronica et al., 2006) and was used to evaluate crispness in this study. Thus, crispness of bean products was significantly affected only in 45% substitutions, demonstrating that although bean addition produced harder and less puffed extrudates, 30% fortification resulted in extrudates with percentage of deformation comparable to corn starch alone (Table 6.1). This is logical by understanding the basic constitution of corn starch and beans, which serves as an argument for most of the physical properties herein discussed. It also agrees with the work of Areas (1992) in a sense that addition of protein to starch-rich flours produces the usual "protein-type" extrudates that are harder and less expanded. Density and breaking strength index were positively correlated ($r$= 0.9, $P$<0.01).

### *6.4.2. Effect of Bean Flour Addition on Some Nutritional Properties of Corn Starch-based Cooked Extrudates*

Bean flour addition, regardless of cultivar, produced a great impact on selected nutritional properties of corn starch-based extrudates (Table 6.3). As whole legume flour contains more proteins than cereal starch (Tharanathan & Mahadevamma, 2003), levels of crude protein increased as a function of increasing rate of bean fortification. It is noteworthy that although we did not characterize the amino acids present our materials, it can be assumed that the amino acid profile of extrudates containing bean flour has changed from almost non-existent (corn starch control) to a relevant source of lysine (Tharanathan & Mahadevamma, 2003).

**Table 6.1.** Physical Properties of Bean Extrudates

| | | Navy | | | Small Red | | |
|---|---|---|---|---|---|---|---|
| | Starch | 15% | 30% | 45% | 15% | 30% | 45% |
| Expansion ratio | 2.54 ± 0.19d | 2.16 ± 0.12c | 1.77 ± 0.11a | 1.7 ± 0.05a | 2.19 ± 0.18c | 1.97 ± 0.12b | 1.78 ± 0.07a |
| Density (g/cc) | 0.11 ± 0.01a | 0.14 ± 0.02b,c | 0.14 ± 0.01b,c | 0.15 ± 0.02b,c | 0.13 ± 0.02a,b | 0.14 ± 0.01b,c | 0.16 ± 0.01c |
| Specific Length (cm/g) | 8.66 ± 0.78a | 10.34 ± 0.96b | 15.11 ± 0.15e | 15.16 ± 0.96e | 10.36 ± 0.65b | 13.67 ± 0.77d | 11.73 ± 0.43c |
| Colour | | | | | | | |
| *L** | 91.08 ± 0.01g | 86.37 ± 0.04f | 84.81 ± 0.08e | 83.5 ± 0.02d | 79.37 ± 0.01c | 75.27 ± 0.14b | 68.9 ± 0.01a |
| *a** | -1.93 ± 0.02a | -0.24 ± 0.01b | 0.10 ± 0.01c | 0.29 ± 0.01d | 2.88 ± 0.01e | 3.78 ± 0.01f | 4.49 ± 0.02g |
| *b** | 10.35 ± 0.09a | 14.17 ± 0.04d | 16.44 ± 0.15f | 17.31 ± 0.07g | 12.22 ± 0.01c | 14.94 ± 0.2e3 | 11.44 ± 0.02b |
| BSI (N/mm$^2$) | 0.16 ± 0.03a | 0.22 ± 0.03b,c | 0.23 ± 0.02b,c | 0.25 ± 0.04c | 0.2 ± 0.03b | 0.21 ± 0.02b | 0.23 ± 0.03b,c |
| dL (Fmax) (mm) | 3.41 ± 0.07f | 2.94 ± 0.06e | 2.22 ± 0.06c | 1.86 ± 0.03b | 2.72 ± 0.08d | 2.61 ± 0.06c | 1.5 ± 0.09a |
| % Deformation | 30.35 ± 2.95c | 30.64 ± 2.74c | 27.43 ± 2.59b,c | 14.16 ± 1.76a | 30.26 ± 3.12c | 27.85 ± 3.82b,c | 24.19 ± 3.22b |

*L**: brightness; *a**: greenness (-) /redness (+); *b**: blueness (-)/yellowness (+).

BSI: breaking strength index – maximal peak force divided by the extrudate cross-sectional area; dL: distance traveled by the shear cell until total breakage.

All the values are Mean ± SD of ten determinations. Data followed by the same character in the same row are not significantly different (*P*>0.05) using a Tukey test comparing all pairs of columns.

**Table 6.2.** Effect of Bean Cultivar on Some Physical and Nutritional Properties of Corn-starch Based Extrudates

| | Bean Flour Concentration (%) | | |
|---|---|---|---|
| | 15 | 30 | 45 |
| Expansion ratio | NS | ** | ** |
| Density | NS | NS | NS |
| Specific length | NS | *** | *** |
| *L** | *** | *** | *** |
| *a** | *** | *** | *** |
| *b** | *** | *** | *** |
| BSI | NS | * | NS |
| dL (Fmax) | *** | *** | *** |
| % Deformation | NS | NS | *** |
| Protein | *** | NS | * |
| TP Raw | *** | *** | *** |
| TP Cooked | *** | *** | *** |
| AOX[1] Raw | *** | *** | *** |
| AOX[1] Cooked | *** | *** | *** |
| AOX[2] Raw | *** | *** | *** |
| AOX[2] Cooked | *** | *** | *** |
| PA Raw | * | * | ** |
| PA Cooked | NS | *** | NS |
| TI Raw | *** | *** | *** |
| TI Cooked | NS | NS | NS |

*L**: brightness; *a**: greenness(-)/redness (+); *b**: blueness (-)/yellowness (+); BSI: breaking strength index – maximal peak force divided by the extrudate cross-sectional area; dL: distance traveled by the shear cell until total breakage; TP: total phenol content; AOX[1]: DPPH$^{\bullet}$ antioxidant activity; AOX[2]: ORAC antioxidant activity; PA: phytic acid; TI: trypsin inhibitors.

NS: no significant effect ($P$>0.05); * $P$<0.05; ** $P$<0.01; ***$P$<0.001 using a two-tail t-test.

In bakery goods, addition of legume flour to cereal-based formulations has proven to positively impact their essential amino acid balance (Koehler et al., 1987; Shehata et al., 1988; Tharanathan & Mahadevamma, 2003). Addition of high protein-high lysine material is known to positively affect the protein quality of cereal foods, since cereal grains are deficient in this amino acid (Pomeranz, 1970). Shehata et al. (1988) showed that addition of 10, 15, or 20% of broad bean flour to Egyptian wheat bread caused a significant ($P<0.01$) increase on protein quality of bread measured by a rat growth study. Yáñez et al. (1989) also demonstrated a slight, non-significant increase on protein efficiency ratio (PER) in breads fortified with 10% of bean flour, however the authors suggested that higher levels of substitution could possibly result in significant increases in bread protein quality.

Total phenols and antioxidant activities determined in the cooked products showed significant variation with respect to bean flour concentration and bean cultivar. Bean flour addition had a positive impact on the levels of these phytochemicals. However, fortification with small red bean flours was to a great extend more effective in producing extrudates with higher potential nutraceutical potential than navy bean flours. As discussed in our previous works (Anton et al., 2008b, c), the colour of dry beans reflects the phytochemical profile of their seed coats, which is composed by compounds such as flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins) (Feenstra, 1960). Since the colour of the seed coats of the cultivars studied are cream white (navy) and dark red (small red), this may explain the differences observed. Recently, the presence of kaempferol, a natural flavonoid with strong antioxidant activity, has been identified in seed coat extracts of red and pinto beans (Hu et al., 2006).

**Table 6.3.** Effect of Extrusion Cooking on Some Nutritional Properties of Corn Starch Added of Bean Flours

| | % | Protein (g/100g) | TP (mg FAE/100g) | | AOX[1] (μmol TE/100g) | | AOX[2] (μmol TE/100g) | |
|---|---|---|---|---|---|---|---|---|
| | | | Raw | Cooked | Raw | Cooked | Raw | Cooked |
| Starch | 100 | 0.3 ± 0.2* | 22.99 ± 2.8* | 20.61 ± 0.7* | 34.04 ± 1.3* | 25.60 ± 1.9*† | 240.17 ± 18.1* | 160.82 ± 7.2*† |
| Navy | 15 | 3.89 ± 0.1a | 31.96 ± 2.9a | 28.27 ± 0.3a† | 62.98 ± 7.1a | 41.42 ± 3.1a† | 289.95 ± 20.4a | 254.36 ± 4.5a† |
| | 30 | 6.88 ± 0.3b | 38.04 ± 0.9b | 36.45 ± 0.9b† | 83.11 ± 3.7b | 71.60 ± 6.2b† | 569.96 ± 55.4b | 508.22 ± 38.2b |
| | 45 | 10.09 ± 0.3c | 52.83 ± 1.3c | 45.96 ± 1.7c† | 140.61 ± 7.5c | 126.23 ± 6.3c† | 617.99 ± 12.9c | 584.46 ± 7.9c† |
| Small Red | 15 | 3.03 ± 0.05a | 119.38 ± 6.6a | 40.94 ± 0.01a† | 528.76 ± 4.1a | 213.93 ± 2a† | 615.28 ± 8.3a | 388.69 ± 24.2a† |
| | 30 | 6.23 ± 0.5b | 217.62 ± 9.4b | 67.09 ± 2.7b† | 1131.12 ± 99.6b | 399.38 ± 4.1b† | 1173.68 ± 84.7b | 1164.93 ± 42.2b |
| | 45 | 9.14 ± 0.6c | 361.56 ± 4.01c | 94.82 ± 1.8c† | 1632.84 ± 20.3c | 642.33 ± 6.1c† | 1750.36 ± 56.4c | 1527.27 ± 121.6c† |

TP: total phenol content; FAE: ferrulic acid equivalents; AOX[1]: DPPH• antioxidant activity; TE: trolox equivalent; AOX[2]: ORAC antioxidant activity.

All the values are Mean ± SD of three/four determinations adjusted to dry matter. Data followed by the same character in the same column, within the same bean flour, are not significantly different (*P*>0.05) using a Tukey test comparing all pairs of columns.

*Significantly different comparing to added bean flours in the same column using a two-tail t-test (*P*<0.05).

† Significantly different comparing to the same raw flour, for the same parameter, using a two-tail t-test (*P*<0.05).

In view of the fact that the authors had not found kaempferol in the seed coat extracts of black and white beans, this may also contribute to justify our findings.

In all cooked extrudates, total phenol levels were significantly ($P$<0.001) correlated to both DPPH$^{\bullet}$ ($r$= 0.98) and ORAC ($r$= 0.98) antioxidant activities. The method for determining antioxidant activity yielded expressively different antioxidant values. Results from the ORAC assay were much higher than the ones from the DPPH$^{\bullet}$ method, yet they were significantly correlated ($r$= 0.98, $P$<0.001). Nonetheless, similar finding have been reported in the literature (Xu et al., 2007; Wang & Ballington, 2007), suggesting that although coloured beans may be an important source of antioxidants, the quantification method of such plays an important role on determining their nutraceutical potential.

### *6.4.3. Effect of Extrusion Cooking on Some Selected Nutritional and Antinutritional Properties of Corn Starch-based Raw Mixtures*

As observed in Table 6.3, extrusion cooking resulted in a significant decrease in the nutraceutical potential of corn starch-bean mixtures. Total phenols were reduced on average 10% in starch and navy bean extrudates, in comparison to the raw mixtures. More significant reductions occurred in small red bean extrudates, which had their total phenols content decreased in approximately 70%. These observations can be extended to what was observed in the levels of both DPPH$^{\bullet}$ and ORAC antioxidant activities after processing of corn starch-bean mixtures. DPPH$^{\bullet}$ antioxidant activity decreased nearly 22% in starch and navy bean extrudates, while antioxidants reached 65% reduction in materials to which small red bean flour was added. ORAC values were also affected by extrusion, however to a lesser extent. Through this method, overall reduction of antioxidant activity was on the range of 1-37%, with no effect of bean cultivar detected through analysis of variance (ANOVA). As mentioned before, different methods for determining antioxidant activity are expected to give different outcomes. Xu and Chang (2008) reported that the oxygen radical absorbance capacity (ORAC) is the only method so far that combines both inhibition time and degree of inhibition into a single quantity (Cao & Prior 1999). The antioxidant reaction mechanism of ORAC is quite different than that of $DPPH^{\bullet}$. ORAC reactions involve a hydrogen atom transfer mechanism, while $DPPH^{\bullet}$ mechanism involves a single electron transfer (Prior et al., 2005). In the ORAC, antioxidant activity provokes the inhibition of the free radical damage to the fluorescent compounds. The different values herein reported may be attributed to the capacity of each method of detecting the antioxidant activity of various compounds. Arnao (2000) speculated that lower DPPH values may be attributed to the interference of other pigments that also absorb at the wavelength used in the $DPPH^{\bullet}$ method (515 nm), such as carotenoids and anthocyanins (Dlamini et al., 2007).

Nonetheless, our results are in accordance with previous works on polyphenols and antioxidant activities of raw and extruded common beans (Alonso et al., 2000; Korus et al., 2007a, b). Korus et al. (2007a) reported that the effect of extrusion on the phenolic content of beans depended on the cultivar. In their study, one bean cultivar showed an increase of 14% in the amount of phenolics in extrudates compared to raw beans, while the other two were decreased by 19 and 21%. They also observed that extrusion at 180°C and 20% moisture of the feed material resulted in the least active materials and decreased antioxidant activity in comparison to the raw flours. Additionally, it is suggested that extrusion may have also promoted the polymerization of phenolic acids and tannin (Remy et al., 2000), thus affecting the extractability of such compounds, and their related reduced antioxidant activities (Dlamini et al., 2007).

On the other hand, antinutritional compounds such as phytic acid and trypsin inhibitors were also significantly reduced by extrusion cooking (Table 6.4). Phytic acid, which was significantly higher in mixtures containing navy beans compared to small red, had an overall reduction of nearly 44% after extrusion. We suggest that during cooking inositol hexaphosphate could have been hydrolyzed to lower molecular weight forms, which is in agreement with the work of Alonso et al. (2000), who reported a significant reduction in phytic acid content in beans submitted to extrusion cooking. Using high performance liquid chromatography (HPLC), these authors revealed that during extrusion, some molecules of inositol hexaphosphate were hydrolysed to penta-, tetra- and triphosphates. Similar reductions in phytic acid levels in whole beans processed under various conditions, including soaking, roasting, autoclaving, and pressure-cooking have been reported (Alonso et al., 2000; ElMaki et al., 2007; Shimelis et al., 2007). It appears that the mechanisms of reduction of phytic acid involved in the processing of whole seeds and flours of beans are quite different. In whole legume seeds it seems to concern the leaching of phytate that occurs during soaking and cooking (Estévez et al., 1991), the phytase activity at a temperature of 40-55 °C that may degrade inositol hexaphosphate to the pentaphosphate or lower molecular weight forms (ElMaki et al., 2007), or the formation of insoluble complexes between phytate and other components during cooking, therefore reducing phytate availability (Kumar et al., 1978).

**Table 6.4.** Effect of Extrusion Cooking on Some Antinutritional Properties of Corn Starch Added of Bean Flours

| | | Phytic Acid (mg/10g) | | Trypsin Inhibitors (TIU/100mg) | |
|---|---|---|---|---|---|
| | (%) | Raw | Cooked | Raw | Cooked |
| Starch | 100 | ND | ND | ND | ND |
| Navy | 15 | 13.77 ± 0.83a | 5.69 ± 0.47a | 331.25 ± 3.1a | ND |
| | 30 | 29.91 ± 1.52b | 9.73 ± 0.46b | 697.92 ± 14.7b | ND |
| | 45 | 41.93 ± 1.89c | 22.19 ± 1.11c | 916.67 ± 6.71c | ND |
| Small Red | 15 | 11.79 ± 0.9a | 5.37 ± 0.62a | 91.88 ± 6.7a | ND |
| | 30 | 25.61 ± 2.07b | 16.69 ± 0.62b | 167.5 ± 5.4b | ND |
| | 45 | 37.91 ± 0.66c | 23.32 ± 1.37c | 244.8 ± 6.6c | ND |

TIU: trypsin inhibitory units; ND: non detectable.

All the values are Mean ± SD of four determinations adjusted to dry matter. Data followed by a different character in the same column, within the same bean flour, are significantly different ($P$<0.05) using a Tukey test comparing all pairs of columns.

All materials were significantly different than their respective raw flours for both phytic acid and trypsin inhibitors ($P$<0.05).

Trypsin inhibitors determined in the raw mixtures were significantly higher in navy beans composites than in small red, and increased as a function of increased level of bean fortification (Table 6.4). Since these inhibitors are thermolabile and their inhibitory activity can be reduced extensively by an appropriate thermal treatment (Alonso et al., 2000; Shimelis et al., 2007; Anton et al., 2008c), low levels were expected in the bean extrudates. In fact, extrusion cooking in the conditions applied was able to reduce trypsin inhibitors by 100% in all samples. This is in agreement with the findings of Alonso et al. (2000) and Balandrán-Quintana et al. (1998), who observed that extrusion cooking was one of the best processing methods for improving the protein quality of legumes.

### 6.5. Conclusions

The effect of bean cultivar was more relevant on the nutritional, rather than physical, properties of corn starch-based extrudates (Table 6.2). Physically, critical differences were observed mainly for colour, which reflects the differences observed in terms of total phenol content and antioxidant activities. Fortification of corn starch with small red bean flour yielded extrudates with higher nutraceutical value compared to navy bean flour substitution. The replacement of corn starch by bean flour, regardless of cultivar, indicates to be feasible at 30%.

Attempts to improve the nutritional and physical properties of bean extrudates by addition of different additives have been reported (Martin-Cabrejas et al., 1999; Berrios et al., 2004; Berrios, 2006). Recently, Berrios et al. (2004) reported that by adding increasing levels of sodium bicarbonate to black bean flour they could produce more expanded bean extrudates. This observation was attributed to the release of $CO_2$ from $NaHCO_3$ facilitated through the heat and moisture provided by the extrusion process. Therefore, it is suggested that bean flour can be incorporated at higher levels in corn starch-based extruded snacks, without great impact on their physical properties, by elaborating processing and formulation with the use of adequate food additives.

At 30% substitution, crude protein was increased 12-fold, while the other chemical compounds studied were also significantly increased. In comparison to raw flours, extrusion significantly decreased concentration of phenolics, antioxidants, TI, and phytic acid.

Further sensory studies and biological trials must be carried out in order to evaluate the acceptability of bean extrudates by a consumer panel and to verify the impact of such foods on animal and human nutrition. Additionally, a modified manufacturing procedure and the application of food additives, such as sodium bicarbonate, may help improve the nutritional profile and the texture of extrudates added at higher levels of bean flour. Nonetheless, it appears that corn starch-bean extrudates have a strong potential to replace regular extruded snacks as a healthier option.

# 7. DISCUSSION

## 7.1. Physical Properties

The physical properties of bean-related materials submitted to different processes have been studied extensively. In Chapter 3 it was observed that thermal treatment of navy and pinto beans caused important changes to their physical properties. Changes in colour were partially attributed to changes in the polyphenolic compounds, ascorbic acid and carotene present in the whole seeds and in the starch fractions. This agrees with the reports of Galvez and Ressureccion (1993) and Shimelis et al. (2006) and is also believed to reflect the changes in levels of phytochemicals and related antioxidant activities as shown in Chapters 3 and 6.

In Chapter 3, the higher viscosities observed for the freeze-dried samples may be due to stronger interactions between starch granules caused by freeze drying, which produced dried seeds with very low moisture contents yet without gelatinizing the starch, thereby affecting the gelatinization process. The high peak viscosities of these samples indicate that the amylose granules were probably packed more compactly, reducing the accessibility of water molecules to the binding sites on the amylose chains of the correspondent starches. Alternatively, the heat treatment likely produced a high degree of gelatinization and subsequent starch degradation, which would result in lower viscosities. Gelatinization of starch has been shown to enhance the extent of hydrolysis in both *in vitro* and *in vivo* studies, and starches in processed foods may be either essentially unchanged, partly or wholly gelatinized, or partly retrograded (Tharanathan &

Mahadevamma, 2003). This treatment seemed to lead to a high level of retrogradation for the bean starches. Sandhu and Singh (2007) reported a negative correlation between retrogradation and peak and final viscosity. Consequently, except for the beverage industry, which could be interested in starches with low final viscosity, the beans submitted to heat treatment would have limited applications in the food industry, as the majority of the existing products demand high viscosity profiles (Su et al., 1997). Prior reports on the effects of heat treatment on the structure and physicochemical properties of cereal and legume starches have suggested that the magnitude of these changes are dependent upon the moisture content during heat treatment and the starch source (Sair, 1967; Lorenz & Kulp, 1981; Hoover & Manuel, 1996). Moreover, temperature had an important impact on seed coat yield, colour and pasting properties, aside the effect on some nutritional properties of the materials herein studied.

Although it was demonstrated that isolation of different fractions of beans is possible, the treatments studied were not economically feasible at a large commercial scale. Thus, for the studies presented in Chapters 4, 5, and 6, whole bean flour was used instead of different fractions of bean cultivars aiming to simulate the processing most typically used in the food industry. In Chapter 4 it was observed that addition of increasing levels of bean flour to wheat flour tortilla formulations caused important, yet expected, changes to some physical properties investigated. The increased water absorption of the bean-wheat doughs could be explained on the basis that most bean proteins are water soluble (Deshpande et al., 1983; Morales-de-Léon et al., 2007) and gluten, the most relevant wheat protein, is mostly water insoluble. Consequently, the higher water absorption of the composites could be related to the high water absorption of the beans (Deshpande et al., 1983). This also supports the observation that dough stability decreased abruptly as bean flour was added. This is logical since beans lack proteins that give wheat dough its viscoelastic properties (Deshpande et al., 1983). Therefore, as the concentration of wheat flour, and consequently its gluten content, was decreased by the addition of bean flour the dough rheological properties were negatively affected. Moreover, dough weakening could also be due to competition between bean proteins and wheat proteins for water or to the possible proteolytic activity in the dry bean flours (Fleming & Sosulski, 1978). Similar behavior has been observed for doughs which include gluten-free flours (Gonzalez-Agramon et al., 1988; Doxastakis et al., 2002; Serna-Saldivar et al., 2004). Physical properties related to the quality of the final tortilla reflected the negative dough rheological properties of bean-wheat composites. In this regard, the negative effect of bean flour addition on textural parameters of tortillas has been reported in tortilla formulations fortified with oat bran and rice bran (Friend et al., 1992) and triticale (Serna-Saldivar et al., 2004). Nonetheless, pinto bean flour has been added to quick bread formulations in levels ranging from 25% to 50% and no significant difference in sensory scores regarding texture was found for any level of substitution (Alani et al., 1989). Dalgetty and Baik (2006) showed a reduction in volume of bread resulted from increased levels of substitution of wheat with legume fibres. It is also hypothesized that bean flour addition is greatly affecting the gluten network of flour tortillas. Tortillas containing 35% of bean flour were less rollable than those added of 0, 15, or 25% bean flour regardless of cultivar, i.e., tortillas broke more easily during rollability test. Similar findings have been reported for whole wheat flour tortillas, suggesting the disruption of the continuous gluten matrix when bran was present (Friend et al., 1992).

In Chapter 4 it was concluded that tortillas fortified with 25% bean flour would have acceptable textural properties. Based on this rationale, in Chapter 5 tortillas to which 25% of pinto bean flour was added had 2 hydrocolloids commonly used in the tortilla industry included in their formulations at 2 different levels, aiming to improve the shelf stability and sensory properties of the end products. Guar gum and sodium carboxymethylcellulose (CMC) were added in order to mimic the gluten properties in the tortilla dough and therefore improve the textural properties of bean tortillas over time. Addition of guar gum produced bean tortillas with a degree of puffiness similar to the wheat control. CMC addition resulted in low quality tortillas, reflecting a low tolerance to mixing and poor dough structure that does not allow puffiness. It has been established that the characteristic fluffy texture of the tortilla depends upon the retention of steam and leavening gases by the gluten matrix (McDonough et al., 1996). In this sense, guar gum was able to partially mimic the gluten functionality, effectively compensating for the weak structure caused by substituting 25% of wheat flour with bean flour. Many types of hydrocolloids have been successfully applied in the production of gluten-free breads (Anton & Arntfield, 2008).

The most important physical parameters determined instrumentally in the shelf stability and quality of tortillas are cohesiveness, rollability and moisture loss (Friend et al., 1992; Friend et al., 1993; Friend et al., 1995; Anton et al., 2008c). The beneficial effects of hydrocolloids in tortilla processing have been discussed by Gurkin (2002). The major functions of hydrocolloids and their interactions were reviewed, and the author concluded that water-binding is the main feature of gums in tortillas. The ability of the large molecules to hold moisture was reported to help prevent staling. Moreover, the interactions between different structures were emphasized. In this regard, the beneficial combination of guar gum with CMC as water-binding agents was cited, confirming their potential as textural improvers. In our shelf stability study, guar gum could visibly retain more water than CMC and the bean control. CMC, nonetheless, allowed similar moisture losses as the bean control.

In the sensory study presented in Chapter 5, overall acceptability, flavour and texture of both bean tortillas had, in general, higher acceptability than the wheat control. No significant difference in these parameters was observed among tortillas evaluated by consumers who usually eat regular white flour bread and tortilas. Nonetheless, texture of bean tortillas with added guar gum was significantly more acceptable ($P<0.001$) by panelists from the "healthy bread and tortillas consumers" group. In this view, instrumental data poorly reflected the data obtained from the sensory evaluation. This makes sense since consumers who are used to consuming multi-grain, whole wheat, high-fibre, and flax bakery goods are more likely to accept different texture, appearance, and flavour in a new nutritious product. Although the wheat control had the best cohesiveness and rollability amongst the tortillas studied, it was the combination of all organoleptic properties that accounted for their acceptability by consumers. Addition of guar gum improved cohesiveness, rollability and moisture loss of bean tortillas over time, however such improvement was not perceived when tortillas were evaluated 24 hours after production.

Chapter 5 demonstrated the technical feasibility of producing acceptable tortillas fortified with 25% of pinto bean flour; however, it is believed that these findings can be extrapolated to different bean cultivars. As observed in Chapter 4, the effect of bean cultivar can be significant in terms of the physical properties of tortillas, nonetheless, at 25% substitutions such effects were not considered critical.

After proving the technical feasibility of producing flour tortillas fortified with 25% of bean flour, the potential of fortifying corn starch-based extruded snacks with flour from 2 bean cultivars was demonstrated in Chapter 6. The negative effect of adding bean flour on the physical properties of extrudates may be attributed to the fact that fibre from beans can rupture cell walls and prevent air bubbles from expanding to their maximum potential (Pérez-Navarrete et al., 2006). Such an argument may also support the fact that navy bean flour fortification produces slightly less expanded products than small red beans. In accordance to what was described in Chapter 4, this may be due to the higher amount of fibre from the seed coats in the flour of navy beans, which is based on the weight of 100 seeds (Navy: 15.17 ± 0.41g; Small red: 27.21 ± 1.83g); when the mass of the seeds is less, the seed coat comprises a larger area relative to one whole seed. Similar behavior has been reported for maize and Lima bean flour extrudates (Pérez-Navarrete et al., 2006). There is solid evidence in the literature that as high-fibre, high-protein materials are added to starch-based extruded products, density is expected to increase (Onwulata et al., 2001; Veronica et al., 2006). Gujska et al. (1991) suggested that the degree of expansion affects the density, fragility and overall texture of extruded products, which can be extended to our findings.

Crispness of bean products was significantly affected only at 45% substitution levels, demonstrating that although bean addition produced harder and less puffed extrudates, 30% fortification resulted in extrudates with acceptable expansion and texture. Nonetheless, attempts to improve the nutritional and physical properties of bean extrudates by addition of different additives have been reported (Martin-Cabrejas et al., 1999; Berrios et al., 2004; Berrios, 2006). Recently, Berrios et al. (2004) reported that by adding increasing levels of sodium bicarbonate to black bean flour they could produce more expanded bean extrudates. This observation was attributed to the release of $CO_2$ from $NaHCO_3$ facilitated through the heat and moisture provided by the extrusion process. Therefore, it is suggested that bean flour should be able to be incorporated at higher levels in corn starch-based extruded snacks, without great impact on their physical properties, by elaborating processing and formulation with the use of adequate food additives. Further studies should be directed towards the use of sodium biocarbonate to confirm this affect on other bean types.

Fortification of non-nutritious snack foods with bean flour appears to be promising. We observed that by using a basic formulation absent of additives and texture improvers, bean flour can be added at levels of 25-30% in flour tortillas and corn starch-based extruded snacks and produce acceptable products. Apart from changes in texture, addition of bean flour caused important changes in the colour of the finished products. This effect was directly related to the bean cultivar used in the fortification. Except for products to which navy bean flour was added, the colour of processed materials was highly affected by incorporating small red, black, or pinto beans. Nonetheless, as there is a market opportunity for introducing new food products with high-nutritional appeal, the health-conscious consumer is likely to accept different organoleptic features such a appearance, flavour, and texture (Anton et al., 2008a). Moreover, colour changes compared to the control product (either flour tortillas or corn starch extrudates) reflected important changes on the nutritional and nutraceutical profile of snacks fortified with coloured bean flours.

### 7.2. Nutritional Properties

Despite the negative effect on some physical properties, fortification of snack foods with flour from different bean cultivars proved to cause a relevant positive impact on selected nutritional properties of flour tortillas and corn starch-based extruded snacks. Since legumes generally contain more proteins than cereals (Tharanathan & Mahadevamma, 2003), addition of bean flour to wheat flour or corn starch increased the protein content of the final product, as observed in Chapters 4 and 6. More relevant is the consequent enhancement on the amino acid profile of wheat tortillas. Although amino acids were not evaluated here, the literature shows that addition of legume flour to wheat flour baked products improves the essential amino acid balance of such foods (Koehler et al., 1987; Shehata et al., 1988; Tharanathan et al., 2003). Addition of high protein-high lysine material is known to positively affect the protein quality of cereal foods, since cereal grains are deficient in this amino acid (Pomeranz, 1970). Shehata et al. (1988) showed that addition of 10, 15, or 20% of broad bean flour to Egyptian wheat bread caused a significant ($P<0.01$) increase on protein quality of bread measured by a rat growth study. Yáñez et al. (1989) also demonstrated a slight, non-significant increase on protein efficiency ratio (PER) in breads fortified with 10% of bean flour; however, the authors suggested that higher levels of substitution could possibly result in significant increases in bread protein quality.

In Chapter 3 it was observed that the highest concentration of phenolic compounds occurred in the seed coats of pinto beans, which is in agreement with the results of Cardador-Martinez et al. (2002) and Rocha-Guzmán et al. (2007). Seed coat colour of dry beans is determined by the presence and amounts of phenolic compounds, such as flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins) (Feenstra, 1960). This may explain the differences observed in chapters 3, 4, and 5; the colour of navy beans seed coats is cream, whereas the seed coats of the other cultivars are dark red (small red), black (black) or predominantly brown (pinto).

In all Chapters reporting total phenols and antioxidant activity, these parameters were always positively and significantly correlated ($P<0.05$). The highest levels of both phenolics and antioxidant activities were observed in materials containing small red, pinto and black bean flours. Recently, the presence of kaempferol, a natural flavonoid with strong antioxidant activity, has been identified in seed coat extracts of red and pinto beans (Hu et al., 2006). In view of the fact that the authors had not found kaempferol in the seed coat extracts of black and white beans, this may also help explain our findings. Madhujith and Shahidi (2005) and Espinosa-Alonso et al. (2006) suggested that variously-coloured dry beans may be an important source of dietary antioxidants. However, the relationships between colour and phenolic content is controversial. While Barampama and Simard (1993) reported results similar to ours in demonstrating an association between colour and phenol content, Guzmán-Maldornado et al. (1996) did not find similar correlations.

Chapters 3 and 6 show different results in terms of the effect of processing on levels of total phenols and antioxidant activity. While in Chapter 3 these parameters increased with the thermal treatment, in Chapter 6 they were dramatically decreased. The findings of Chapter 3 were explained on the grounds of previous suggestions that some phenolic compounds and their conjugated forms can be converted from one form to another during various technological processes (Stewart et al., 2000; Turkmen et al., 2005). These data indicated that thermal processing of beans under the specified conditions may liberate phenolic compounds and their derivatives from the cell walls. The liberated compounds may then contribute higher antioxidant potential when they are considered as dietary antioxidants. Fernández et al. (1982) also found a significant increase in phenolic compounds after cooking beans under high pressure. Analyzing the level of bioactive compounds in cereal grains before and after hydrothermal processing, Zieliński et al. (2001) demonstrated an increase of up to 300% in the content of phenolic acids after extrusion cooking. The antioxidant activity of grape seed extracts has also been shown to be affected by heating conditions, showing a higher reducing power after various heat-treatments (Kim et al., 2006). However, some reports have shown a reduction of total phenolics in beans submitted to different heat treatments (Alonso et al., 2000; Abd El-Hady & Habiba, 2003; Rocha-Guzmán et al., 2007).

In Chapter 6 bean materials were exposed to extrusion cooking, which led to different and more complex physico-chemical changes compared to heating hydrated beans for different exposure times as shown in Chapter 3. These results are in accordance with previous works on polyphenols and antioxidant activities of raw and extruded common beans (Alonso et al., 2000; Korus et al., 2007a; Korus et al., 2007b). Korus et al. (2007a) reported that the effect of extrusion on the phenolic content of beans depended on the cultivar. In their study, one bean cultivar showed an increase of 14% in the amount of phenolics in extrudates compared to raw beans, while the other two were decreased by 19 and 21%. They also observed that extrusion at 180°C and 20% moisture of the feed material resulted in the least active materials and decreased antioxidant activity in comparison to raw flours. Additionally, it is suggested that extrusion may have also promoted the polymerization of phenolic acids and tannin (Remy et al., 2000), thus affecting the extractability of such compounds, and their related reduced antioxidant activities (Dlamini et al., 2007). This combination of factors may have contributed to the reduction in phenolics and antioxidant activity seen in this study.

Phytic acid levels were unaffected in Chapter 3, however important reductions were observed in Chapters 4 and 6. Soaking and heating as demonstrated in Chapter 3 were not effective in reducing phytic acid levels. Nonetheless, Hurrell et al. (2002) reported that high temperatures, such as those used in bread making, must be applied to degrade phytic acid. In Chapters 4 and 6, it was found that a consistent reduction in phytic acid levels occurred in composite flours processed into tortillas or extruded snacks. Such reductions may be explained on the basis that during cooking inositol hexaphosphate could have been hydrolyzed to lower molecular weight forms, which is in agreement with the work of Alonso et al. (2000), who reported a significant reduction in phytic acid content in beans submitted to extrusion cooking. Through analysis performed by high performance liquid chromatography (HPLC), these authors revealed that during extrusion, some molecules of inositol hexaphosphate were hydrolysed to penta-, tetra- and triphosphates. Similar reductions in phytic acid levels in whole beans processed under various conditions, including soaking, roasting, autoclaving, and pressure-cooking have been reported (Alonso et al., 2000; ElMaki et al., 2007; Shimelis & Rakshit, 2007), however a direct comparison with these studies is difficult since the biochemical reactions involved in the processing of whole seeds and flour of legumes occur in a different manner (Alonso et al., 2000). However, mechanisms that may explain the reduction of phytic acid content during processing of whole legume seeds concern the leaching of phytate that occurs during soaking and cooking (Estévez et al., 1991), the phytase activity at a temperature of 40-55 °C that may degrade inositol hexaphosphate to the pentaphosphate or lower molecular weight forms (ElMaki et al., 2007), or the formation of insoluble complexes between phytate and other components during cooking, therefore reducing phytate availability (Kumar et al., 1978).

Trypsin inhibitors are thermolabile and their inhibitory activity can be reduced considerably by an appropriate thermal treatment (Alonso et al., 2000; Shimelis & Rakshit, 2007). In tortillas, detailed in Chapter 4, our results are in agreement with the findings of Estévez et al. (1991), who found that levels of trypsin inhibitors were reduced from 57 to 62% in beans soaked for 16 h at room temperature and cooked for 60 minutes. This study reported that the cooked bean samples showed *in vitro* protein digestibility of nearly 90%, as well as good net protein ratios (3.2 in comparison to 4.2 of casein). It has been suggested that inactivation of trypsin inhibitors depends on the physical state of the material. Carvalho and Sgarbieri (1997) reported that bean flours submitted to soaking and autoclaving had significantly lower levels of inactivation than whole beans. In

Chapter 4, our findings are comparable with bean flours soaked for 12 h and autoclaved at 121°C for 5 and 20 min resulting in 55 and 65% reductions, respectively. Since inactivation of trypsin inhibitors in bean tortillas has not been complete, it appears that a thermal treatment longer than that usually applied in flour tortillas manufacturing may be necessary. Nevertheless, based on the levels of substitution studied and on the degree of inactivation reached, it seems that the present bean tortillas would be safe for human nutrition.

In Chapter 6, extrusion cooking in the conditions applied was able to reduce trypsin inhibitors by 100%. This is in agreement with the findings of Alonso et al. (2000) and Balandrán-Quintana et al (1998), who observed that extrusion cooking was one of the best processing methods for improving the protein quality of legumes.

# 8. CONCLUSIONS

The study of treatments aimed to facilitate the dehulling of beans indicates that, depending on the temperature and moisture conditions, important nutritional and physical properties of beans can be manipulated to yield end products with specific characteristics. The impact of the various pre-dehulling treatments on nutraceutical value and corresponding characteristics of the whole seeds demonstrated that research must be continued in this area, to achieve feasible processes for the preparation of high quality products.

The effect of bean cultivar on some physical and nutritional properties of wheat- and corn-based snacks was more significant for substitutions higher than 25%. Fortification at 25-30% produced acceptable textural parameters and improved nutritional profile in comparison to the wheat and corn starch controls. Colour and levels of phenolics and antioxidant activity were not changed to a great extent in materials to which navy bean flour was added, however these parameters were significantly affected for the other type of beans. Levels of antinutritional compounds were significantly reduced at all levels of substitution, indicating that bean snacks could be safely used for human nutrition.

Further sensory studies and biological trials must be carried out in order to evaluate the acceptability of bean snacks by a larger consumer panel and to verify the impact of such foods on animal and human nutrition. Nonetheless, the overall analysis appears to indicate that production of snacks enriched with bean flour is feasible at the substitution rate of 30% and that their consumption can play an important role in the maintenance of a healthy life style.

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